FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES	**10648-0001-0 PCT**
DESIGNATED/ELECTED OFFICE (DO/EO/US)	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
CONCERNING A FILING UNDER 35 U.S.C. 371	**09/446109**

INTERNATIONAL APPLICATION NO. **PCT/AU98/00490**	INTERNATIONAL FILING DATE **25 JUNE 1998**	PRIORITY DATE CLAIMED **25 JUNE 1997**

TITLE OF INVENTION

CYCLIC AGONISTS AND ANTAGONISTS OF C5a RECEPTORS AND G PROTEIN-COUPLED RECEPTORS

APPLICANT(S) FOR DO/EO/US

David FAIRLIE, et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))

    a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).

    b. ☒ has been transmitted by the International Bureau.

    c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☒ A copy of the International Search Report (PCT/ISA/210).

8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))

    a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).

    b. ☐ have been transmitted by the International Bureau.

    c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.

    d. ☒ have not been made and will not be made.

9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).

11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).

12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

15. ☒ A **FIRST** preliminary amendment.

    A **SECOND** or **SUBSEQUENT** preliminary amendment.

16. ☐ A substitute specification.

17. ☐ A change of power of attorney and/or address letter.

18. ☐ Certificate of Mailing by Express Mail

19. ☒ Other items or information:

> Notice of Priority
> PCT/IB/304
> Written Opinon (2)
> Request For Consideration of Documents Cited in International Search Report

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) 09/446109	INTERNATIONAL APPLICATION NO. PCT/AU98/00490	ATTORNEY'S DOCKET NUMBER 10648-0001-0 PCT

20. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

CALCULATIONS PTO USE ONLY

☐ Search Report has been prepared by the EPO or JPO **$840.00**

☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) **$670.00**

☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$760.00**

☒ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO **$970.00**

☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$96.00**

ENTER APPROPRIATE BASIC FEE AMOUNT = | **$970.00** |

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). | **$130.00** |

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	23 - 20 =	3	x $18.00	**$54.00**	
Independent claims	2 - 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims (check if applicable).			☐	**$0.00**	

TOTAL OF ABOVE CALCULATIONS = $1,154.00

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐ **$0.00**

SUBTOTAL = $1,154.00

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + **$0.00**

TOTAL NATIONAL FEE = $1,154.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐ **$0.00**

TOTAL FEES ENCLOSED = $1,154.00

Amount to be: refunded	$
charged	$

☒ A check in the amount of **$1,154.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

WILLIAM E. BEAUMONT
REGISTRATION NUMBER 30,996

SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

December 23, 1999
DATE
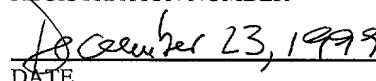
Docket No.: 10648-0001-0 PCT

<u>IN THE UNITED STATES PATENT & TRADEMARK OFFICE</u>

IN RE APPLICATION OF: :

DAVID FAIRLIE ET AL   : ATTN: APPLICATION DIVISION

SERIAL NO: NEW APPLICATION   :
            (BASED ON PCT/AU98/00490)

FILED: HEREWITH   :

FOR: CYCLIC AGONISTS AND
      ANTAGONISTS OF C5A
      RECEPTORS AND G PROTEIN-
      COUPLED RECEPTORS

<u>PRELIMINARY AMENDMENT</u>

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

    Prior to examination on the merits, please amend the above-identified application as

follows.

<u>IN THE CLAIMS</u>

    Please delete Claims 24-32.

    Please amend the claims as follows.

    Claim 3, line 1, delete "or Claim 2".

    Claim 4, line 1, replace "3" with --1--.

    Claim 5, lines 1-2, replace "any one of Claims 1 to 4" with --Claim 1--.

    Claim 7, lines 1-2, replace "any one of claims 1 to 4" with --Claim 1--.

Claim 8, lines 1-2, replace "any one of Claims 1 to 4" with --Claim 1--.

Claim 9, lines 1-2, replace "any one of Claims 1 to 7" with --Claim 1--.

Claim 13, line 1, replace "any one of Claims 10 to 12" with --Claim 10--.

Claim 14, lines 1-2, replace "any one of Claims 3 and 10 to 13" with --Claim 13--.

Claim 17, line 1, delete "or Claim 16".

Claim 18, lines 1-2, replace "any one of Claims 15 to 17" with --Claim 15--.

Claim 20, line 2, replace "any one of Claims 1 to 19" with --Claim 1--.

Claim 21, line 4, replace "any one of Claims 1 to 19" with --Claim 1--.

REMARKS

Claims 1-23 are active in this application.

The claims have been amended to remove multiple dependencies. No new matter is believed to have been added to this application by these amendments.

Applicants submit that the present application is ready for examination on the merits.

Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
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23 DEC

CYCLIC AGONISTS AND ANTAGONISTS OF C5a RECEPTORS
AND G PROTEIN-COUPLED RECEPTORS

This invention relates to novel cyclic compounds
which have the ability to modulate the activity of G
protein-coupled receptors. The invention provides both
agonists and antagonists. In preferred embodiments, the
invention provides cyclic peptidic and cyclic or non-cyclic
non-peptidic antagonists or agonists of C5a. The compounds
of the invention are both potent and selective, and are
useful in the treatment of a variety of inflammatory
conditions.

BACKGROUND OF THE INVENTION

Activation of human complement, a system of
plasma proteins involved in immunological defence against
infection and injury, contributes significantly to the
pathogenesis of numerous acute and chronic diseases. In
particular, the complement protein C5a has been extensively
investigated. For general reviews, see Whaley (1987), and
Sim (1993). Table 1 provides a summary of known roles of
C5a in disease.

During host defence, the complement system of
plasma proteins initiates inflammatory and cellular immune
responses to stimuli such as infectious organisms
(bacteria, viruses, parasites), chemical or physical
injury, radiation or neoplasia. Complement is activated
through a complex cascade of interrelated proteolytic
events that produce multiple bioactive peptides, some of
which (eg. anaphylatoxins C3a and C5a) interact with
cellular components to propagate inflammatory processes.
Complement activation, either by the classical pathway,
after antigen-antibody (Ag/Ab) binding, or by the antibody-
independent alternate pathway, ends with a terminal
sequence in which protein C5 is proteolytically cleaved by
C5 convertase to C5a and C5b. The latter facilitates
assembly of a "membrane attack complex" that punches holes

- 2 -

in membranes of target cells such as bacteria, leading to
leakage, lysis and cell death. Steps in the cascade are
tightly regulated to avoid stepwise amplification of
proteolysis by sequentially formed proteases. If these
5 regulatory mechanisms become inefficient, protracted
activation of complement can result, causing enhanced
inflammatory responses as in autoimmune diseases.

Although the broad features of the complement
system and its activation are known, mechanistic details
10 remain poorly understood. A principal and very potent
mediator of inflammatory responses is the plasma
glycoprotein C5a, which interacts with specific surface
receptors (C5aR) on mast cells, neutrophils, monocytes,
macrophages, non-myeloid cells, and vascular endothelial
15 cells (Gerard and Gerard, 1994). C5aR is a G protein-
coupled receptor with seven transmembrane helices (Gerard
and Gerard, 1991). This receptor is one of the rhodopsin
superfamily of GTP-linked binding proteins, but differs
from rhodopsin receptors in that the receptor and G protein
20 are linked prior to activation.

G protein-coupled receptors are prevalent
throughout the human body, comprising approximately 80% of
known cellular receptor types, and mediate signal
transduction across the cell membrane for a very wide range
25 of endogenous ligands. They participate in a diverse array
of physiological and pathophysiological processes,
including, but not limited to those associated with
cardiovascular, central and peripheral nervous system,
reproductive, metabolic, digestive, immunoinflammatory, and
30 growth disorders, as well as other cell-regulatory and
proliferative disorders. Agents, both agonists and
antagonists, which selectively modulate functions of G
protein-coupled receptors have important therapeutic
applications.

35   C5a is one of the most potent chemotactic agents
known, and recruits neutrophils and macrophages to sites of
injury, alters their morphology; induces degranulation;

- 3 -

increases calcium mobilisation, vascular permeability
(oedema) and neutrophil adhesiveness; contracts smooth
muscle; stimulates release of inflammatory mediators
(including histamine, TNF-α, IL-1, IL-6, IL-8,

5   prostaglandins, leukotrienes) and lysosomal enzymes;
promotes formation of oxygen radicals; and enhances
antibody production (Gerard and Gerard, 1994).
Overexpresion or underregulation of C5a is implicated in
the pathogenesis of immunoinflammatory conditions such as

10   rheumatoid arthritis, adult respiratory distress syndrome
(ARDS), systemic lupus erythematosus, tissue graft
rejection, ischaemic heart disease, reperfusion injury,
septic shock, psoriasis, gingivitis, atherosclerosis,
Alzheimer's disease, lung injury and extracorporeal post-

15   dialysis syndrome, and in a variety of other conditions, as
summarised in Table 1.

Table 1

The Role of C5a in Disease

Condition/disease	C5a levels	C5aR expression	Details
allergy	++		allergen challenge leads to nasal symptoms and increased C5a levels
Alzheimer's disease	++	++	up-regulation of the receptor in reactive astrocytes, microglia and endothelial cells in the CNS, complement system activated by ß-amyloid
ARDS/respiratory distress	++		
Behcet's disease	++		levels highest just prior to ocular attack
bronchial asthma	++		
capillary leak syndrome	++		

Condition/disease	C5a levels	C5aR expression	Details
chronic lung disease	++		Increased C5a levels in pulmonary effluent fluid from mechanically ventilated infants with chronic lung disease
Churg-Strauss			hypersensitivity of granulocytes to C5a
cystic fibrosis			generation of C5a/effects on PMNs
decompression stress	++		increased C5a levels during saturation diving
diabetes type I	++		C5a generated during onset; circulating monocytes in newly diagnosed type 1 diabetes patients are activated
Familial Mediterranean fever			lack of C5a inactivator
Guillain-Barre	++		CSF levels elevated
ischaemic disease states/ myocardial infarct			migration of monocytes into myocardium after reperfusion. Damage prevented with sCR1

Condition/disease	C5a levels	C5aR expression	Details
Kimura's disease			humoral factor up-regulates the response of PMNs to C5a
Multiple Sclerosis		++	increased expression of the receptor on foamy macrophages in acute and chronic MS and fibrous astrocytes in chronic MS
Meningitis			C5a induces experimental meningitis; PMN accumulation seen in the CSF
pancreatitis	++		
post-dialysis syndrome	++	-	C5a generated via complement activation by tubing material, C5aR levels decreased on PMNs & monocytes in chronic state
preeclampsia/ HELLP	++		C5a levels elevated at delivery
psoriasis	++		C5a levels high in scales
reperfusion injury	++		inhibited by C5 antibody
retinitis	++		C5a detected in vitreous humor

Condition/disease	C5a levels	C5aR expression	Details
Rheumatoid arthritis	++		elevated concentration of C5a found in synovial fluid (5-fold) and plasma (3-fold)
Severe congenital neutropenia		-	
transplant/graft rejection	++		monoclonal antibodies block the damage seen with xenogenic transplant; increased levels of C5a seen in the plasma and urine of patients with renal graft rejection

- 8 -

New agents which limit the pro-inflammatory actions of C5a have potential for inhibiting chronic inflammation, and its accompanying pain and tissue damage. For these reasons, molecules which prevent C5a binding to

5   its receptors are useful for treating chronic inflammatory disorders driven by complement activation. Importantly, such compounds provide valuable new insights to mechanisms of complement-mediated immunity.

In another context, agonists of C5a receptors or

10   other G protein-coupled receptors may also be found to have therapeutic properties in conditions either where the G protein-coupled receptor can be used as a recognition site for drug delivery, or where triggering of such receptors can be used to stimulate some aspect of the human immune

15   system, for example in the treatment of cancers, viral or parasitic infections.

One approach to the development of agonists or antagonists of C5a is through receptor-based design, using knowledge of the three-dimensional structures of C5a, its

20   receptor C5aR, and the interactions between them. The structure of the receptor is unknown. The solution structure of human C5a, a 74 amino acid peptide that is highly cationic and N-glycosylated with a 3 kDa carbohydrate at Asn64, has been determined and is

25   essentially a 4-helix bundle. The C-terminal end (residues 65-74, $C5a_{65-74}$) was found to be unstructured (Zuiderweg et al, 1989) and this conformational flexibility in the C-terminus has made structure-function studies extremely difficult to interpret.

30   C5a has a highly ordered N-terminal core domain (residues 1-64; $C5a_{1-64}$), consisting of a compact antiparallel 4-helix bundle (residues 4-12, 18-26, 32-39, 46-63) connected by loops (13-17, 27-31, 40-45), and further stabilised by 3 disulphide bonds (C21-Cys47, Cys22-

35   Cys54, Cys34-Cys55).

Although the structure of the C5a receptor, C5aR, is unknown, the C5a-binding subunit of human monocyte-

- 9 -

derived C5aR has been cloned and identified as a G protein-
coupled receptor with transmembrane helices (Gerard and
Gerard, 1991). Interactions between C5a and C5aR have been
the subject of many investigations which, in summary,

5   suggest that C5a binds via a two-site mechanism in which
the N-terminal core domain of C5a is involved in receptor-
recognition and binding, while the C-terminus is
responsible for receptor activation. This mechanism is
illustrated schematically in Figure 1. The C-terminal

10 "effector" region alone possesses all the information
necessary for signal transduction, and is thought to bind
in the receptor's interhelical region (Siciliano et al,
1994; deMartino et al, 1995).

An N-terminal interhelical positively-charged

15 region of C5a is responsible for receptor recognition and
binding, and binds to a negatively-charged extracellular
domain of C5aR (site 1), while the C-terminal "effector"
region of C5a is thought to bind with the interhelical
region of the receptor (site 2), and is responsible for

20 receptor activation leading to signal transduction
(Siciliano et al, 1994).

Numerous short peptide derivatives of the C-
terminus of C5a have been found to be agonists of C5a
(Kawai et al, 1991; Kawai et al, 1992; Kohl et al, 1993;

25 Drapeau et al, 1993; Ember et al, 1992; Sanderson et al,
1994; Sanderson et al, 1995; Finch et al, 1997; Tempero et
al, 1997; Konteatis et al, 1994; DeMartino et al, 1995).
The structures of some of these agonists are shown in Table
2 below (compounds 1-6). High molecular weight polypeptide

30 inhibitors of the action of C5a at its receptor, such as
monoclonal antibodies to the C5a receptor, are also known
(Morgan et al, 1992).

A small molecule, N-methylphenylalanine-lysine-
proline-D-cyclohexylalanine-tryptophan-D-arginine (7, MeF-

35 K-P-dCha-W-R), is a full antagonist of the C5a receptor,
with no agonist activity when tested on isolated cellular
membranes (Konteatis et al, 1994) or intact whole cells.

AMENDED SHEET (Article 34) (IPEA/AU)

- 10 -

This hexapeptide was developed by modifications of the
agonist NMe-F-K-P-dCha-L-r, in which the molecule was
progressively substituted at leucine residues with
substituents of increasing size (Cha, F, Nph and W). This
5   had the effect of reducing agonist activity. Receptor-
binding assays, performed on isolated human neutrophil
membranes, showed that the antagonist had only 0.04%
relative affinity of C5a for the receptor (Konteatis *et al*,
1994). A key feature of these reports is the definition of
10 the binding of **7** to the C5a receptor. These authors state
that the C-terminal arginine is essential for receptor
binding and antagonist activity. This is also the case in
all the reports of agonist activity by small peptide
analogues of the C-terminus of C5a. However, for the
15 antagonist **7**, the authors go further and state that
            "the C-terminal carboxylate is an essential
            requirement for antagonist activity and receptor
            binding."
They proposed that the requirement of the
20 carboxylate is probably the result of its specific
interaction with an arginine (Arg 206) in the receptor (De
Martino *et al*, 1995). This idea was supported by a great
reduction in receptor-affinity for an analogue of **7** in
which the D-arginine ($NH_2-CH(CO_2H)-(CH_2)_3NHC(:NH)NH_2$) was
25 replaced by agmatine ($NH_2-CH_2-(CH_2)_3NHC(:NH)NH_2$). In
summary, De Martino *et al* claim that the D-arginine
interacts via its guanidinium side chain with a negatively-
charged amino acid side chain in the receptor. A second
interaction between the negatively-charged C-terminal
30 carboxylate of **7** and a positively-charged side chain
residue in the receptor is also thought to occur.
            We have now determined the solution structure of
this hexapeptide **7** and several analogues, and have
surprisingly found that in fact a terminal carboxylate
35 group is not required for binding to C5aR or for antagonist
activity, and that instead an unusual hitherto unrecognised
structural feature, a turn conformation, is responsible for

- 11 -

C5a antagonist or agonist binding and activity. The hexapeptide and several new structurally related antagonists have been examined for both their receptor-binding affinities and antagonist activity, using intact
5   polymorphonuclear (PMN) cells. Our results show the hitherto unknown specific structural requirement for the binding of C5a antagonists or agonists to the C5a receptor, which we believe to be common to ligands for the G protein-coupled receptor family. Our establishment of this
10   specific structural requirement has enabled us to design and develop improved molecular probes of the complement system and of C5a-based drugs, and to design small molecules that target other G protein-coupled receptors, which are becoming increasingly recognised as important
15   drug targets due to their crucial roles in signal transduction (G protein-coupled Receptors, IBC Biomedical Library Series, 1996).

Thus our results have enabled us to design constrained structural templates which enable hydrophobic
20   groups to be assembled into a hydrophobic array for interaction with a G protein-coupled receptor, for example at Site 2 of the C5a receptor illustrated in Figure 1. Such templates or scaffolds, which may be cyclic or acyclic, have not heretofore been suggested for modulators
25   of the activity of C5a receptors or other G protein-coupled receptors.

SUMMARY OF THE INVENTION

The invention provides cyclic and non-cyclic
30   modulators of the activity of G-protein-coupled receptors.

According to a first aspect, the invention provides a compound which is an antagonist, of a G protein-coupled receptor, which has no agonist activity, and which has a cyclic or constrained acyclic structure adapted to
35   provide a framework of approximate dimensions as follows:

- 12 -

Structure I
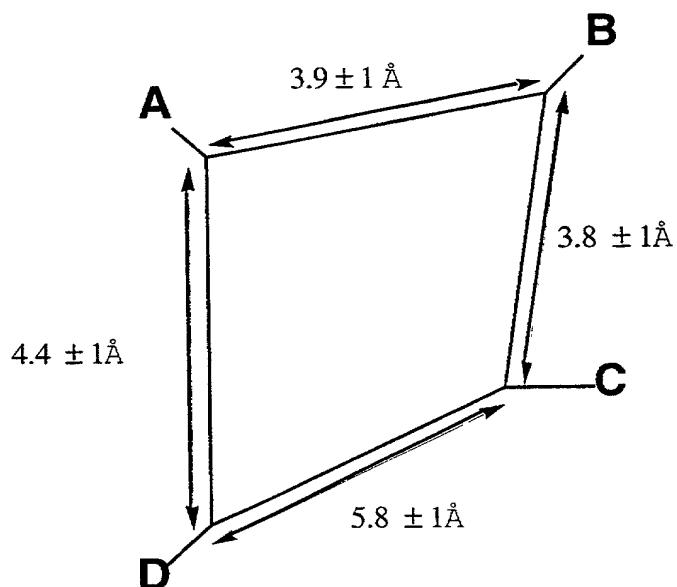
where the numerals refer to distances between C_α carbons of amino acids or their analogues or derivatives, and A, B, C and D are not necessarily on adjacent amino acids, or analogues or derivatives thereof; and

where the critical amino acid side chains are designated by A, B, C and D, or are as defined below;

A is any common or uncommon, basic, charged amino acid side chain which serves to position a positively charged group in this position, including, but not limited to the following side chains and other mimetics of arginine side chains:

$$-(CH_2)_n-\overset{\overset{\displaystyle NHR^1}{|}}{\underset{\underset{\displaystyle H}{N}}{C}}\!\!\overset{\oplus}{=}\!S \; ,$$

$$-(CH_2)_n-\overset{\overset{\displaystyle NHR^1}{|}}{\underset{\underset{\displaystyle H}{N}}{C}}\!\!\overset{\oplus}{=}\!NH_2 \; , \qquad -(CH_2)_n-\overset{\overset{\displaystyle NHR^1}{|}}{\underset{\underset{\displaystyle H}{N}}{C}}\!=\!S \; ,$$

$$-(CH_2)_nO-\overset{\overset{\displaystyle NHR^1}{|}}{\underset{\underset{\displaystyle H}{N}}{C}}\!\!\overset{\oplus}{=}\!NH_2 \; , \qquad -(CH_2)_n-S-\overset{NHR^1}{\underset{}{C}}\!\!\overset{\oplus}{=}\!NH_2 \; , \qquad -(CH_2)_n-\overset{\overset{\displaystyle NHR^1}{|}}{\underset{\underset{\displaystyle H}{N}}{C}}\!=\!X \; .$$

$$-(CH_2)_n-\overset{\oplus}{N}H_3$$
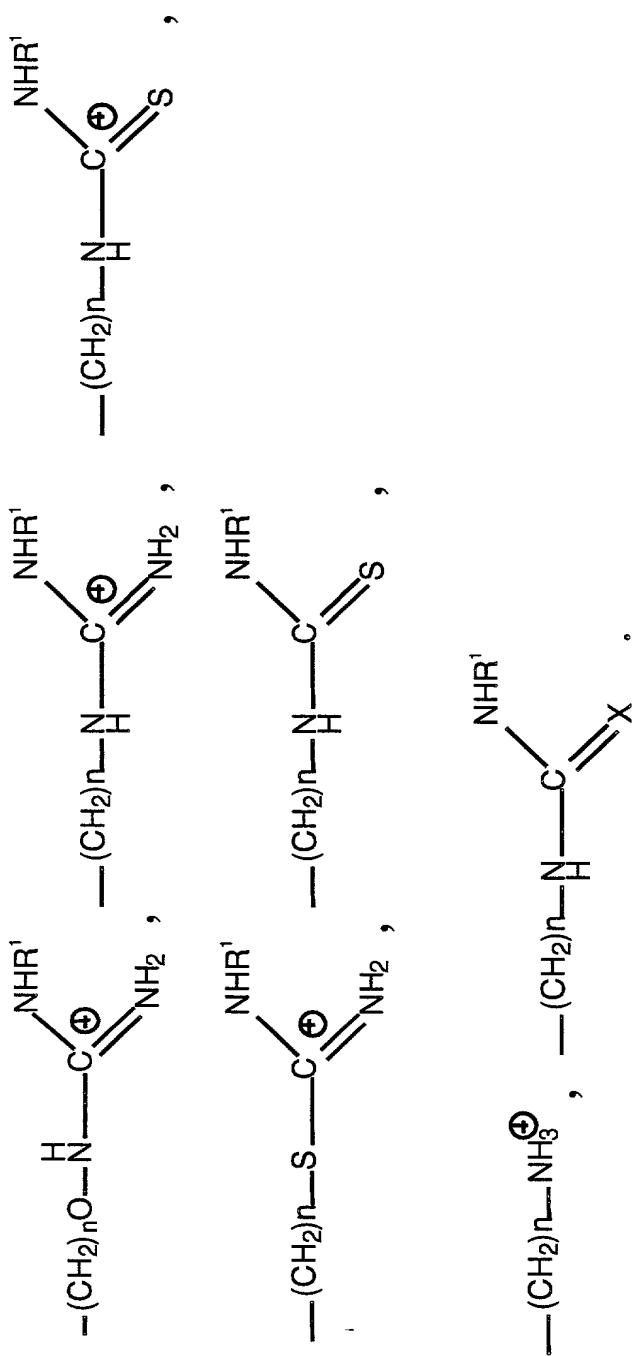
where

X is NCN, NNO$_2$, CHNO$_2$ or NSO$_2$NH$_2$;

n is an integer from 1 to 4, and

R is H or an alkyl, aryl, CN, NH$_2$ or OH group .

5   B is any common or uncommon aromatic amino acid
side chain which serves to position an aromatic side-chain
group in this position, including but not limited to the
indole, indole methyl, benzyl, phenyl, naphthyl, naphthyl
methyl, cinnamyl group, or any other derivatives of these
10   aromatic groups;

C is any common or uncommon hydrophobic amino
acid side chain which serves to position any alkyl,
aromatic or other group in this position, including, but
not limited to D- or L-cyclohexyl alanine (Cha), leucine,
15   valine, isoleucine, phenylalanine, tryptophan, or methionin

D is any common or uncommon aromatic amino acid
which serves to position an aromatic side-chain in this
position, and has the structure:
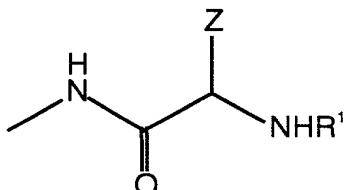
20

where Z includes but is not limited to indole, indole
methyl, benzyl, benzene, naphthyl, naphthyl methyl, or any
other derivatives of these aromatic groups, and

25   R^1 is H or any alkyl, aromatic, acyl or aromatic-
acyl group including, but not limited to methyl, ethyl,
propyl, butyl, -CO-CH$_2$CH$_3$, -CO-CH$_3$, -CO-CH$_2$CH$_2$CH$_3$, -CO-CH$_2$Ph,
or -CO-Ph.

Preferably the G protein-coupled receptor is a
30   C5a receptor.

Other cyclic or constrained acyclic molecules,
which may be peptidic or non-peptide in nature, can
similarly be envisaged to support groups such as A, B, C

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and D for interaction with a C5a receptor or other G
protein-coupled receptor.

In one preferred embodiment, the compound has
antagonist activity against C5aR, has no C5a agonist
5   activity, and has the general formula:

Structure II
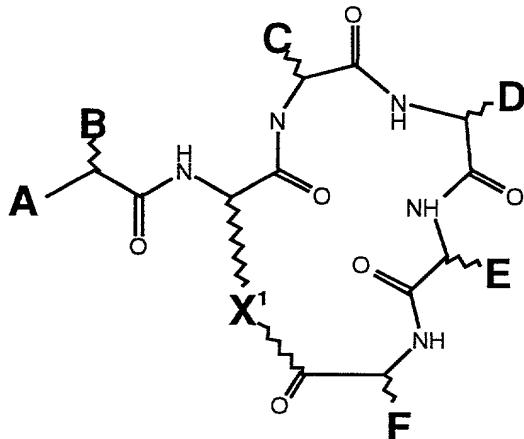
10

where   A is H, alkyl, aryl, NH_2, NHalkyl, $N(alkyl)_2$,
NHaryl or NHacyl;OH, Oalkyl, Oaryl.
15   B is an alkyl, aryl, phenyl, benzyl, naphthyl or
indole group, or the side chain of a D- or L-amino acid
selected from phenylalanine, homophenylalanine, tryptophan,
homotryptophan, tyrosine, and homotyrosine;
         C is the side chain of a D-, L- or homo-amino
20   acid selected from the group consisting of proline,
alanine, leucine, valine, isoleucine, arginine, histidine,
aspartate, glutamate, glutamine, asparagine, lysine,
tyrosine, phenylalanine, cyclohexylalanine, norleucine,
tryptophan, cysteine and methionine;
25   D is the side chain of a D- or L-amino acid
selected from the group consisting of cyclohexylalanine,
homocyclohexylalanine, leucine, norleucine, homoleucine,
homonorleucine and tryptophan;

E is the side chain of a D- or L-amino acid selected from the group consisting of tryptophan and homotryptophan;

F is the side chain of a D- or L-amino acid selected from the group consisting of arginine, homoarginine, lysine and homolysine; and

X^1 is $-(CH_2)_nNH-$ or $(CH_2)_n-S-$, where n is an integer of from 1 to 4, preferably 2 or 3, $-(CH_2)_2O-$, $-(CH_2)_3O-$, $-(CH_2)_3-$, $-(CH_2)_4-$, or $-CH_2COCHRNH-$, where R is the side chain of any common or uncommon amino acid.

For the purposes of this specification, the term "alkyl" is to be taken to mean a straight, branched, or cyclic, substituted or unsubstituted alkyl chain of 1 to 6, preferably 1 to 4 carbons. Most preferably the alkyl group is a methyl group. The term "acyl" is to be taken to mean a substituted or unsubstituted acyl of 1 to 6, preferably 1 to 4 carbon atoms. Most preferably the acyl group is acetyl. The term "aryl" is to be understood to mean a substituted or unsubstituted homocyclic or heterocyclic aryl group, in which the ring preferably has 5 or 6 members.

A "common" amino acid is a L-amino acid selected from the group consisting of glycine, leucine, isoleucine, valine, alanine, phenylalanine, tyrosine, tryptophan, aspartate, asparagine, glutamate, glutamine, cysteine, methionine, arginine, lysine, proline, serine, threonine and histidine.

An "uncommon" amino acid includes, but is not restricted to, D-amino acids, homo-amino acids, N-alkyl amino acids, dehydroamino acids, aromatic amino acids (other than phenylalanine, tyrosine and tryptophan), ortho-, meta- or para-aminobenzoic acid, ornithine, citrulline, norleucine, γ-glutamic acid, aminobutyric acid and α,α-disubstituted amino acids.

For the purposes of this specification it will be clearly understood that the word "comprising" means

"including but not limited to", and that the word
"comprises" has a corresponding meaning.

According to a second aspect, of the invention
provides a compound which is an agonist of G protein-
coupled receptors, and which has structure III
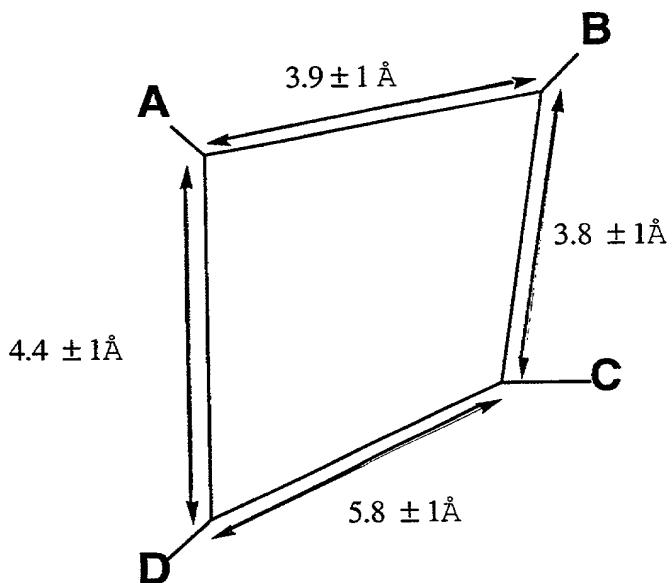
Structure III

where the numerals refer to distances between C_α
carbons of amino acids or their analogues or derivatives,
and A, B, C and D are not necessarily on adjacent amino
acids, or analogues or derivatives thereof; and

where B is a non-aromatic amino acid, and is
preferably the D- or L-form of alanine, leucine, valine,
norleucine, glutamic acid, aspartic acid, methionine,
cysteine, isoleucine, serine or threonine,

and A, C and D are as defined above.

Preferably the compound is of structure IV,

- 18 -

Structure IV
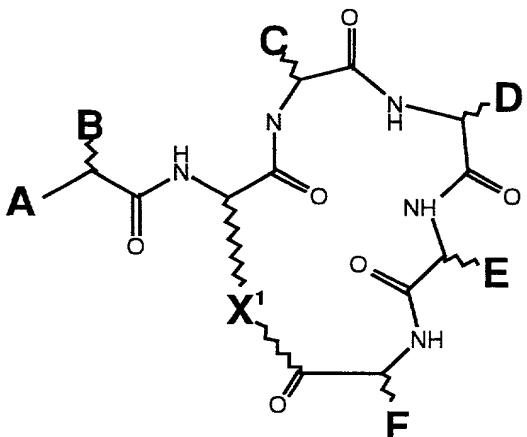
5   where E is any amino acid other than tryptophan
and homotryptophan, for example D- or L- forms of alanine,
leucine, valine, norleucine, phenylalanine, glutamic acid,
aspartic acid, methionine, cysteine, isoleucine, serine,
threonine, and F and X^1 are as defined in Structure II.

10   Preferably the compound is an agonist of C5a.

     According to a third aspect, the invention
provides a composition, comprising a compound according to
the invention together with a pharmaceutically-acceptable
carrier or excipient.

15   The compositions of the invention may be
formulated for oral or parenteral use, but oral
formulations are preferred. It is expected that most if
not all compounds of the invention will be stable in the
presence of digestive enzymes. Such stability can readily

20   be tested by routine methods known to those skilled in the
art.

     Suitable formulations for administration by any
desired route may be prepared by standard methods, for
example by reference to well-known textbooks such as

25   Remington; The Science and Practice of Pharmacy, Vol. II,
1995 (19th edition), A.R. Gennaro (ed), Mack Publishing
Company, Easton, Pennsylvania, or Australian Prescription
Products Guide, Vol. 1, 1995 (24th edition) J. Thomas (ed),

WO 99/00406PCT/AU98/00490

- 19 -

Australian Pharmaceutical Publishing Company Ltd, Victoria,
Australia.

In a fourth aspect, the invention provides a
method of treatment of a pathological condition mediated by
5   a G protein-coupled receptor, comprising the step of
administering an effective amount of a compound of the
invention to a mammal in need of such treatment.

Preferably the condition mediated by a G protein-
coupled receptor is a condition mediated by a C5a receptor,
10   and more preferably involves overexpression or
underregulation of C5a. Such conditions include but are
not limited to rheumatoid arthritis, adult respiratory
distress syndrome (ARDS), systemic lupus erythematosus,
tissue graft rejection, ischaemic heart disease,
15   reperfusion injury, septic shock, psoriasis, gingivitis,
atherosclerosis, Alzheimer's disease, lung injury and
extracorporeal post-dialysis syndrome.

While the invention is not in any way restricted
to the treatment of any particular animal or species, it is
20   particularly contemplated that the compounds of the
invention will be useful in medical treatment of humans,
and will also be useful in veterinary treatment,
particularly of companion animals such as cats and dogs,
livestock such as cattle, horses and sheep, and zoo
25   animals, including large bovids, felids, ungulates and
canids.

The compounds may be administered at any suitable
dose and by any suitable route. Oral administration is
preferred because of its greater convenience and
30   acceptability. The effective dose will depend on the
nature of the condition to be treated, and the age, weight,
and underlying state of health of the individual treatment.
This will be at the discretion of the attending physician
or veterinarian. Suitable dosage levels may readily be
35   determined by trial and error experimentation, using
methods which are well known in the art.

- 20 -

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a diagrammatic representation of the two-site model for binding of C5a to its G protein-coupled receptor, C5aR. The black rods represent α-helical regions, and the open cylinders represent the transmembrane helices. Sites 1 and 2 are indicated on the figure.

Figure 2 shows stacked plots of ^{1}H-NMR spectra, showing time-dependent decay of amide NH resonances for Trp (8.10 ppm) and D-Cha (7.90 ppm) residues of 7 in d_6-DMSO containing D_2O after 10 minutes (bottom plot) and then 25, 40, 55, 70, 130, 190, 250, 385 and 520 minutes.

Figure 3 shows backbone C, N, O atoms of twenty lowest energy minimized NMR structures of 7 in d_6-DMSO at 24°C) .

Figure 4 shows a schematic representation of H-bonding in the structure of 7 from proton NMR spectra in d_6-DMSO.

Figure 5a shows (a) receptor binding, as indicated by inhibition of binding of ^{125}I-C5a to human PMNs by 7 (●); 8 (Δ); 9 (▲); 12 (O) .

Figure 5b shows C5a antagonist potency as inhibition of myeloperoxidase (MPO) release from human PMNs by : 7 (■, n= 9) and 12 (▲, n=4) .   Figure 5c shows C5aR binding and antagonist potencies of 7, 15 and 17.

A-C show the effect of increasing concentrations (top to bottom) of C5a antagonists inhibiting myeloperoxidase release in human PMNs (n=3 in A-C).

A: 7 at 0, 0.1, 0.3, 1.0 μM (top to bottom)

B: 15 at 0, 0.1, 0.03, 0.1 μM (top to bottom)

C: 17 at 0, 0.01, 0.03, 0.1 μM (top to bottom)

D: Comparative affinities for PMN C5qR receptor. Inhibition of binding of ^{125}I-C5a to human PMNs by 7 (top), 15 (middle), 17 (bottom). All data are means ± SEM.

Figure 6 shows receptor binding of cyclic C5a antagonists, as shown by inhibition of binding of ^{125}I-C5a to human PMNs (n=5) .

- 21 -

Figure 7 shows superimposed structures of **7**
(light, NMR structure) and **12** (dark, computer modelled
structure). Phe and Trp side chains are omitted from **12** for
clarity.

5   Figure 8 shows inhibition of C5a-induced
neutropenia in Wistar rats by the cyclic antagonist F-
[OPdChaWR] given i.v. at 1mg/kg. Results shown from n=3 in
each group, *P<0.05 compared to C5a-treated group only.
Results are expressed as mean ± SEM.

10   Figure 9 shows inhibition of LPS-induced
neutropenia and changes in haematocrit induced by the
cyclic antagonist F-[OPdChaWR] (0.03-10mg/kg, i.v., 10 min
prior to lipopolysaccharide [LPS]) in Wistar rats.
Abscissa: time after LPS (1 mg/kg i.v. injection).

15 Ordinate: per cent change in haematocrit (A) value or level
of circulating polymorphonuclear (PMN) leukocytes (B)
compared to time zero.

Figure 10 shows inhibition of carrageenan-induced
(Wistar) rat paw oedema by cyclic antagonist (3D35) AcF-

20 [OPdChaWr] (1 mg/kg single dose i.p. given 30 min prior to
carrageenan). Results shown from 4 rats /group, mean ± SEM.
Ordinate: percent change in paw volume. Abscissa: time
(mins) after carrageenan injection

25 <u>DETAILED DESCRIPTION OF THE INVENTION</u>

The invention will now be described by way of
reference only to the following general methods and
experimental examples, and to the figures. Abbreviations
used herein are as follows:

30 BOP   benzotriazole-1-yl-oxy-tris-(dimethylamino)-
phosphonium hexafluorophosphate

D-Cha   D-cyclohexylamine

DIPEA   diisopropylethylamine

DMF   dimethylformamide

35 DMSO   dimethylsulphoxide

HBTU   O-benzotriazole N',N',N',N'-tetramethyluronium
hexafluorophosphate;

- 22 -

LPS   lipopolysaccharide

PMN   polymorphonuclear granulocyte

RMSD   root mean square deviation

RP-HPLC   reverse phase-high performance liquid

5   chromatography

TFA   trifluoroacetic acid;

Throughout the specification conventional single-
letter and three-letter codes are used to represent amino
10   acids.

General Methods

Protected amino acids and resins were obtained
from Novabiochem. TFA, DIPEA and DMF (peptide synthesis
15   grade) were purchased from Auspep. All other materials were
reagent grade unless otherwise stated. Preparative scale
reverse-phase HPLC separations were performed on a Vydac
C18 reverse-phase column (2.2 x 25 cm), and analytical
reverse-phase HPLC separations were performed on a Waters
20   Delta-Pak PrepPak C18 reverse-phase column (0.8 x 10 cm),
using gradient mixtures of solvent A = water/0.1% TFA and
solvent B = water 10%/acetonitrile 90%, 0.09% TFA. The
molecular weight of the peptides was determined by
electrospray mass spectrometry recorded on a triple
25   quadrupole mass spectrometer (PE SCIEX API III), as
described elsewhere (Haviland et al, 1995). ^{1}H-NMR spectra
were recorded on either a Bruker ARX 500 MHz or a Varian
Unity 400 spectrometer. Proton assignments were determined
by 2D NMR experiments (DFCOSY, TOCSY, NOESY).

30   Non-peptidic compounds were synthesized using
conventional organic chemical methods. Compounds were
analysed by ^{1}H-NMR spectroscopy and by mass spectrometry.

Peptide synthesis

35   Some representative peptide syntheses are now
given. Linear peptide sequences were assembled by manual
step-wise solid-phase peptide synthesis with HBTU

activation and DIEA *in situ* neutralisation. Boc chemistry
was employed for temporary N^α-protection of amino acids
with two 1 min treatments with TFA for Boc group removal.
The peptides were fully deprotected and cleaved by
treatment with liquid HF (10 ml; p-cresol (1 ml); -5°C; 1-
2 hr). Analytical HPLC (gradient; 0% B to 50% B over
40 min): **7**, Rt = 32.0 min, $[M+H]^+$(calc.) = 900.5,
$[M+H]^+$(exper.) = 900.7; **8**, Rt = 32.2 min,
$[M+H]^+$(calc.) = 899.6, $[M+H]^+$(exper.) = 899.7;
9, Rt = 30.0 min, $[M+H]^+$(calc.) = 900.5, $[M+H]^+$(exper.) =
900.7; **10**, Rt = 23.8 min, $[M+H]^+$(calc.) = 860.5,
$[M+H]^+$(exper.) = 860.5.

Structures for the peptides are shown in Table 4
below.

- 24 -

a)   Synthesis of cycle 11

This is a general method used for the synthesis
of a wide range of cyclic antagonists covered by this
patent. For example, in the case of cycle **11**, its linear

5   precursor peptide was synthesised by Fmoc chemistry using
HBTU/DIEA activation on an Fmoc-D-Arg(Mtr)-Wang resin.
Fmoc group removal was effected using two 1 min treatments
with 50% piperidine/DMF. Cleavage and deprotection using
95% TFA/2.5% TIPS/2.5% H_2O gave the Mtr-protected peptide,

10   which was purified by RP-HPLC. Cyclization of the
protected, purified peptide using 3eq BOP and 10eq DIEA at
a 1 mM concentration in DMF stirring for 15 hr gave the
cyclised product, which was fully deprotected using 1M
TMSBr in TFA. A final RP-HPLC purification gave the

15   desired peptide in yields of 50% for the cyclisation. Rt =
37.7 min, $[M+H]^+$(calc.) = 910.5, $[M+H]^+$(exper.) = 910.7.

b)   Synthesis of cycle 12

Cyclization of the cleaved and fully deprotected·

20   peptide was achieved by stirring a 1mM solution in DMF with
3eq BOP and 10 eq pyridine as base for 15hr. A final RP-
HPLC purification gave the desired peptide in yields of 22%
for the cyclization. Rt = 37.3 min, $[M+H]^+$(calc.) = 896.5,
$[M+H]^+$(exper.) = 896.5.

25

NMR Structure Determination

^{1}H-NMR spectra were recorded for compound **7** (3 mg
in 750 μl d_6-DMSO, δ 2.50) referenced to solvent on a
Varian Unity 400 spectrometer at 24°C. Two dimensional

30   ^{1}H-NMR NOESY (relaxation delay 2.0s, mix time 50-300 ms),
DFQ- COSY and TOCSY (mixing time 75ms) experiments were
acquired and recorded in phase sensitive mode. Acquisition
times = 0.186 s, spectral width = 5500Hz, number of complex
points (t_1 dimension) = 1024 for all experiments. Data was

35   zero-filled and Fourier transformed to 1024 real points in
both dimensions.

- 25 -

NMR data was processed using TRIAD software
(Tripos Assoc.) on a Silicon Graphics Indy work station.
2D NOE cross peaks were integrated and characterised into
strong (1.8-2.5Å), medium (2.3-3.5Å) and weak (3.3-5.0Å).
5   Preliminary three-dimensional structures were calculated
from upper and lower distance limit files using Diana 2.8
(69 distance constraints, including 27 for adjacent
residues and 6 further away) with the redundant dihedral
angle constraints (REDAC) strategy. Upper and lower
10   distance constraints were accurately calculated using
MARDIGRAS. At this stage the peptide was examined for
possible hydrogen bonds, and these were added as distance
constraints. The 50 lowest energy Diana structures were
subjected to restrained molecular dynamics (RMD) and energy
15   minimisation (REM). Initially, REM consisted of a 50 step
steepest descent followed by 100 step conjugate gradient
minimisation. RMD was performed by simulated heating of
the structures to 300K for 1ps, followed by 500K for 1ps.
The temperature was gradually lowered to 300K over 2ps and
20   finally for 2ps at 200K. REM was performed again with a 50
step steepest descent, 200 step conjugate gradient followed
by a 300 step Powell minimisation. The final structures
were examined to obtain a mean pairwise rms difference over
the backbone heavy atoms (N, Cα and C). Twenty of the 50
25   structures had a mean rmsd < 0.5 Å for all backbone atoms
(O, N, C).

Molecular Modelling

A model of cycle **12**, shown in Figure 7, was
30   created from the NMR structure of **7** by deleting all NMR
constraints, fusing the ornithine side chain amine to the
C-terminal carboxylate of d-Arg to form an amide, and
minimising using Powell forcefield (1000 iterations). The
modelled structure was then superimposed on the NMR
35   structure with an rmsd 0.224Å.

- 26 -

Receptor-Binding Assay

Assays were performed with fresh human PMNs, isolated as previously described (Sanderson *et al*, 1995), using a buffer of 50 mM HEPES, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5%
5   bovine serum albumin, 0.1% bacitracin and 100 µM phenylmethylsulfonyl fluoride (PMSF). In assays performed at 4°C, buffer, unlabelled human recombinant C5a (Sigma) or peptide, Hunter/Bolton labelled ^{125}I-C5a (~ 20 pM) (New England Nuclear, MA) and PMNs (0.2×10^6) were added
10 sequentially to a Millipore Multiscreen assay plate (HV 0.45) having a final volume of 200 µL/well. After incubation for 60 min at 4°C, the samples were filtered and the plate washed once with buffer. Filters were dried, punched and counted in an LKB gamma counter. Non-specific
15 binding was assessed by the inclusion of 1 mM peptide or 100 nM C5a which typically resulted in 10-15% total binding.

Data was analysed using non-linear regression and statistics with Dunnett post test.
20

Myeloperoxidase Release

Cells were isolated as previously described (Sanderson *et al*, 1995) and incubated with cytochalasin B (5µg/mL, 15 min, 37°C). Hank's Balanced Salt solution
25 containing 0.15% gelatin and peptide was added on to a 96 well plate (total volume 100 µL/well), followed by 25 µL cells ($4x10^6$/mL). To assess the capacity of each peptide to antagonise C5a, cells were incubated for 5 min at 37°C with each peptide, followed by addition of C5a (100 nM) and
30 further incubation for 5 min. Then 50 µL of sodium phosphate (0.1M, pH 6.8) was added to each well, the plate was cooled to room temperature, and 25 µL of a fresh mixture of equal volumes of dimethoxybenzidine (5.7 mg/mL) and H_2O_2 (0.51%) was added to each well. The reaction was
35 stopped at 10 min by addition of 2% sodium azide. Absorbances were measured at 450 nm in a Bioscan 450 plate

- 27 -

reader, corrected for control values (no peptide), and
analysed by non-linear regression.

In Vivo Assays of Anti-Inflammatory Activity

5   The following well-known in vivo assay systems
may be used to assess the anti-inflammatory activity of
compounds of the invention. All assay data are analysed
using non-linear regression analysis and Student's t-test,
analysis of variance, with $p < 0.05$ as the threshold level of
10   significance.

(a) Carrageenan Paw Oedema

          Anaesthetised (i.p. ketamine & xylazine) Wistar
rats (150-200g) or mice were injected with sterilised air
15   (20ml day 1, 10ml day 4) into the subcutaneous tissue of
the back. The cavity can be used after 6 days, whereupon
carrageenan (2ml, 1% w/w in 0.9% saline) was injected into
the air pouch and exudate was collected after 10 hr. Test
compounds are administered daily after Day 6 and their
20   anti-inflammatory effects assayed by differential counting
of cells in the air-pouch exudate. Animals were killed at
appropriate times after injection and 2ml 0.9% saline was
used to lavage the cavity, lavage fluids were transferred
to heparinised tube and cells were counted with a
25   haemocytometer and Diff-Quik stained cytocentrifuged
preparation.
          Alternatively, a routine carrageenan paw oedema
was developed in Wistar rats by administering a pedal
injection of carrageenan to elicit oedema which is visible
30   in 2h and maximised in 4h. Test compounds are given 40
min before inflammagen and evaluated by microcaliper
measurements of paws after 2 & 4 hr. See Fairlie,D.P. et
al (1987). Also see Walker and Whitehouse (1978).

35   **(b) Adjuvant Arthritis.**
          Adjuvant arthritis was induced in rats (3
strains) either microbially (injection of heat-killed

- 28 -

Mycobacterium tuberculosis) or chemically (with avridine) by inoculation with the arthritogenic adjuvant co-administered with oily vehicles (Freund's adjuvants) in the tail base. (See Whitehouse, M. W., Handbook of Animal
5 Models for the Rheumatic Diseases, Eds. Greenwald, R. A.; Diamond, H. S.; Vol. 1, pp. 3-16, CRC Press) ·

Within 13 days the adjuvant arthritis is manifested by local inflammation and ulceration in the tail, gross swelling of all four paws, inflammatory lesions
10 in paws and ears, weight loss and fever. These symptoms, which are similar to those of inflammatory disease in humans (Winter and Nuss, 1966), can be alleviated by agents such as indomethacin or cyclosporin which also show beneficial effects in man (*eg.* Ward and Cloud, 1966).
15 Without drug treatment at Day 14, arthritic rats had hypertrophy of the paws, reduced albumin but raised acute phase reaction proteins in serum, and depressed hepatic metabolism of xenobiotics as indicated by prolonged barbiturate-induced sleeping times.
20 To assess activity, compounds were administered for 4 days orally ($\leq$10mg/kg/day) or i.p. from Days 10-13 following inoculation with arthritogen (Day 0). The inflammation was either not visible or very significantly reduced in rear or front paws as assessed by microcaliper
25 measurements of paw thickness and tail volume, as well as by gross inspection of inflammatory lesions. Animals are sacrificed by cervical dislocation on Day 18 unless arthritis signs are absent, whereupon duration of observations is continued with special permission from the
30 Ethics committees. Experiments are staggered to maximise throughput and allow early comparisons between compounds. This routine assay is well-accepted as identifying anti-inflammatory agents for use in humans.

Example 1   Structure-Activity Relationship of C5a
              Agonists

     We have focussed on the C-terminal residues of
C5a, in order to explore structure-activity relationships
in the search for peptide sequences with potent agonist
activity. Many of these peptides are full agonists
relative to C5a, but have markedly lower potency (Sanderson
et al, 1994, 1995; Finch *et al*, 1997). Our initial
structure-activity investigations have been particularly
informative. Mutating the decapeptide C-terminus of C5a
(**1**, C5a$_{65-74}$, ISHKDMQLGR) twice with I$_{65}$Y and H$_{67}$F (*eg*. **2**)
led to enhancement of agonist potency by about 2 orders of
magnitude. These results are summarised in Table 2.
Analyses of Ramachandran plots and 2D NMR spectra for
compound **2** suggested that certain structural features,
namely a twisted "helix-like" backbone conformation for
residues 65-69 and a ß-turn for residues 71-74, might be
responsible for activity. These preliminary results
provided some insight to structural requirements for tight
binding to a C5a receptor.

Table 2

Pharmacological Activity of C5a Agonist Analogues*

Peptide No.	Peptide	Fetal Artery EC_{50} (μM)	PMN Enzyme Release EC_{50} (μM)	Binding Affinity IC_{50} (μM)
1	C5a$_{65-74}$ (ISHKDMQLGR)	>1000	>1000	>1000
2	YSFKDMQLGR	9.6	92	1.3
3	YSFKDMPLaR	0.5	72	3.7
4	YSFKPMPLaR	0.2	4.1	6.0
5	C5a$_{37-46}$-ahxYSFKPMPLaR	0.06	5.9	0.7
6	C5a$_{12-20}$-ahxYSFKPMPLaR	0.08	0.7	0.07
	C5a	0.02	0.03	0.0006

* Finch et al, 1997

- 31 -

Compounds **4**, **5** and **6** in Table 2 are the highest affinity small C5a agonists so far known, with up to 25% C5a potency in human fetal artery, 5% C5a potency in human PMN enzyme release assays and 1% C5a affinity for PMN C5aR
5   (Finch et al., 1997). For the PMN receptor, these compounds have up to 100-fold higher apparent affinity than any small molecule previously described in the literature.

The "high" affinities (70nM-6μM) of these agonist analogues for C5aR in intact PMN cells have enabled us to
10 identify a common topographical feature in peptide agonists that correlates with expression of spasmogenic activities and enzyme-release assays in human PMNs. This preferred backbone conformation is a type II ß-turn.

The small size of these agonist peptides makes
15 them amenable to synthetic modification to optimise their affinities, activities, and bioavailabilities, and hence useful as mechanistic probes of receptor activation.

Example 2   NMR Structure of C5a Antagonist
20   We used two dimensional nuclear magnetic resonance spectroscopy to determine the three dimensional structure of **7** and found that while there is no discernible structure in water, there is evidence of a stable gamma-turn structure in dimethylsulfoxide.
25   The 1D ^{1}H-NMR spectrum of peptide **7** in d$_6$-DMSO at 24°C shows 4 distinct resonances for amide-NH protons, as summarized in Table 3. To establish their possible involvement in intramolecular hydrogen bonds, a deuterium exchange experiment was performed by adding a 10-fold
30 excess of D$_2$O to the solution. Two of the amide-NH doublets disappeared immediately, along with resonances attributable to the N-terminal methylamine protons. However, the other two amide NH resonances, as well as a broad resonance at approximately 8.05 ppm, persisted for up
35 to 6.5 hours (Figure 2). These three slowly-exchanging protons are assigned to the amide NHs of Trp and d-Cha and the side chain amine of Lys, the slow exchange behaviour

- 32 -

being characteristic of hydrogen-bonding. The amine
assignment was established from the TOCSY spectrumm where
cross peaks were observed between the protonated amine and
the ε, δ and γ CH$_2$ protons. A temperature dependence study
(20-60°C) of the amide-NH chemical shifts ($\Delta\delta/T$ = 2.5
ppb/deg, dCha-NH; 6 ppb/deg, Trp-NH; 6.5 ppb, Lys-NH;
8.7ppb, Arg-NH) unambiguously confirmed the involvement of
the dCha-NH only in intramolecular hydrogen bonding.

Table 3

^{1}H-NMR Assignments^a for **7** in d_6-DMSO

Residue	^{b}HN	Hα	Hβ	Hγ	Others
MePhe	–	4.06	3.09, 3.06	–	^{c}7.17, 7.29; ^{d}2.46; ^{f}8.98
Lys	8.83	4.54	1.74, 1.55	1.32	^{e}1.51; ^{f}2.74, ^{g}7.76 (NH$_2$)
Pro	–	4.30	2.084, 1.74	1.88, 1.78	^{e}3.61, ^{f}3.48
d-Cha	7.91	4.35	1.19, 1.06	0.76	^{e}1.43, 1.08; ^{f}1.61, 1.58; 0.73
Trp	8.01	4.65	3.11, 2.94	–	^{c}6.97,7.06,7.13,7.32,7.65; ^{g}10.80
d-Arg	8.44	4.20	1.73, 1.58	1.42	^{e}3.08; ^{g}7.60

a   Referenced to residual d$_5$-DMSO at 2.50 ppm.

b   Amide NHs, $^3J_{NH-CaH}$ values (Hz):7.91 (Lys), 7.77 (d-Arg), 8.34 (Trp), 8.53 (d-Cha).

c   Aromatics

d   N-Me.

e   Hδ.

f   Hε

g   NH/NH$_2$ amine.

5

10

- 34 -

A series of 2D ^{1}H-NMR spectra were measured for **7** at 24°C in d_6-DMSO to determine the three-dimensional structure. TOCSY and DFQ-COSY experiments were used to identify residue types, while sequential assignments were

5   made from analysis of NOESY data. From a series of 100 structures generated from NOESY data, fifty of the lowest energy structures were subjected to restrained molecular dynamics (200K-500K) and energy minimised. A set of 20 calculated structures with a root mean square deviation

10   (rmsd) < 0.5Å (backbone atoms) are superimposed in Figure 3, and clearly depict a turn conformation.

In combination, the NMR constraint data, $^3J_{NH-C\alpha H}$ values, deuterium exchange and temperature dependence data establish an unusual turn structure for hexapeptide **7** which

15   is constrained by up to three hydrogen bonds, as shown in Figure 4. The evidence is very strong for *one intramolecular hydrogen bond* from dCha-NH....OC-Lys (2.72 Å, N-H..O angle 157°, C=O...H angle 84°), forming a 7-membered ring that defines an inverse γ-turn. The

20   dChaNH-O-TrpNH angle is 56.4°. The deuterium exchange data and NMR constraint data together point to a *second intramolecular hydrogen bond* Trp-NH....OC-Lys (3.31 Å, N-H..O angle 159°, CO...H angle 137.3°) forming a 10-membered ring characteristic of a β-turn. The φ and ψ angles

25   (ϕ_2=-58.4°, ψ_2=62.0°; ϕ=96.6°, ψ_3=16.6°) most closely match a type II β-turn (Bandekar, 1993; Hutchinson and Thornton, 1994) which is distorted by the presence of the γ-turn wholly *within* the β-turn.

To our knowledge this is the first example of an

30   intramolecular hydrogen bond between residues within a β-turn, although there are many examples of hydrogen bonds between a residue within the "10 membered ring" of a β-turn and a residue outside of it (Bandekar, 1993). *A third hydrogen bond* (2.76Å, N-H...O angle 160.3°), between the

35   side-chain amine of Lys and the C-terminal carboxylate, is suggested by the NMR constraint data, by slow NH/ND exchange and by detection of a weak NOE between Lys-

- 35 -

NH...Trp-αCH$_2$. This may further constrain the molecule
into the observed turn conformation. Such ion-pairing is
common in dipolar aprotic solvents such as
dimethylsulphoxide and may also be relevant in a
5 hydrophobic protein environment.

NMR solution structures have also been determined
for several of the cyclic antagonists described in the
following examples, and show that in each case the type II
β-turn is preserved and stabilized by the cyclic structure.
10 The constraining β and γ turns proposed in the
linear peptide **7** have parallels in cyclic peptides. We
have previously detected overlapping β and γ turns in a
cyclic octapeptide from ascidiacyclamide (Abbenante *et al*,
1996). Combinations of a β- and γ-turn have also been
15 found in the backbones of cyclic penta- and hexapeptides,
particularly those containing alternating D- and L-amino
acids (Marraud and Aubry 1996; Fairlie *et al*, 1995; Kessler
et al, 1995; Stradley *et al*, 1990). For example a type II
β-turn and an inverse γ-turn have been identified in cyclic
20 antagonists c-(D- Glu-Ala-D-allo-Ile-Leu-D-Trp] (Ihara *et*
al, 1991; Coles *et al*, 1993; Ihara *et al*, 1992; Bean *et al*,
1994) and c-(D-Asp-Pro-D-Val-Leu-D-Trp) (Bean *et al*, 1994)
for endothelin receptors, and in members of the rhodopsin
family of G protein-coupled receptors with seven
25 transmembrane domains (X.-M.Cheng *et al*, 1994). In the
latter case, as in **7**, an inverse γ-turn forms between
residues (Asp-CO.....Val-NH, Lys-CO....dCha-NH) that flank
the proline.

30 <u>Example 3   Structure-Activity Relationships *In Vitro*</u>
We also examined the receptor-binding and
antagonist activity of the hexapeptide **7** for comparison
with our new compounds. The previous report by Konteatis
et al (1994) concerned the ability of **7** to compete with C5a
35 binding to receptors on isolated PMN membranes (IC$_{50}$
70 nM), which is not necessarily physiologically relevant.
We examined competition between **7** and C5a using intact PMN

- 36 -

cells, and found that, under these conditions, **7** binds with
much lower receptor affinity of IC_{50} 1.8 μM. We confirmed
that **7** is a full antagonist with no agonist properties.
These results are summarized in Figure 5a and Table 4. The

5   *relative* affinity (ratio) of **7** for the C5aR in intact PMNs
in our assays was similar to that previously reported for
isolated PMN membranes.

We have also found that **7** shows antagonist
activity against both C5a (Figure 5b) and a C-terminal

10   agonist decapeptide analogue **4** (YSFKPMPLaR) (Finch *et al*,
1997) of the C-terminus $C5a_{65-74}$, suggesting that it acts
on site 2 of the receptor. Compounds **7** and **4** have similar
μM affinity for the receptor C5aR on intact
polymorphonuclear leukocytes, as shown in Table 4.

15   A new discovery from the data in Table 4 is the
linear correlation between the log of binding affinities
and the log of antagonist potencies for these Site 2
antagonists (compounds **7-12**, Table 4). The importance of
this linear relationship is that since receptor affinity

20   and antagonist activity are directly proportional, the
experimentally simpler approach of measuring receptor
binding may be used to estimate the antagonist activity for
such small compounds, provided that there is no evidence of
agonist activity.

25

Table 4

Receptor-Binding Affinities[a] and Antagonist Activities[b] in Human PMNs

Compound	Receptor Affinity[a] IC_{50} (μM)	Antagonist Potency[b] IC_{50} (μM)	Agonist Activity[c]
7 MeFKP(dCha)Wr	1.8 (15)	0.085 (9)	No
8 MeFKP(dCha)Wr-CONH$_2$	14 (5)	0.5 (3)	No
9 MeFKP(dCha)WR	11 (5)	0.7 (3)	No
10 MeFKPLWR	144 (1)	>1000 (3)	nd
11 Ac-F-[KP(dCha)Wr]	3.2 (40	0.090 (5)	No
12 Ac-F-[OP(dCha)Wr]	0.28 (6)	0.012 (4)	No
4 YSFKPMPLaR	6.0[d]	-	Yes
1 C5a65-74, ISHKDMQLGR	>1000[e]	-	-
C5a	0.0008 (9)	-	Yes

Number of experiments in parenthesis. Corrected for amino acid content.

Square brackets indicate cyclic portion.

nd = not determined

[a] 50% reduction in binding of ^{125}I-C5a to intact human PMNs

[b] 50% reduction in myeloperoxidase secretion from human PMNs mediated by 100 nM C5a

[c] Agonist activity in dose range 0.1 nM-1 mM

[d] Finch et al, 1997; [e] Kawai et al, 1991

- 38 -

It has previously been proposed that the C-
terminus of C5a and of agonist peptides is essential for
activity, due to its interaction with a positively-charged
Arg206 of the receptor (DeMartino et al, 1995). We confirm
5   here that the C-terminal carboxylate is indeed important
for activity (**8** vs. **7**), but wondered whether the origin of
this effect might be due to hydrogen bonding between the
carboxylate anion and the positively charged amine side
chain of Lys. Conversion to the amide (**8**) certainly reduces
10   both receptor-affinity and antagonist activity
approximately 5-fold. Changing chirality of the Arg-Cα (**9**
vs. **7**) causes a similar reduction in activity, and
replacing dCha with the less bulky Leu residue (**10**) is also
detrimental to receptor binding. However, potency is
15   recovered for cyclic compounds **11** and **12**, in which an amide
bond is tolerated at the C-terminus, consistent with the
structural interpretation above that the advantage of the
carboxylate in **7** may be associated with intramolecular
hydrogen bonding. The replacement of this hydrogen bond in
20   **7** with a covalent amide bond in **11** and **12** more effectively
stabilizes the turn conformation.

Figure 5C compares C5aR binding and antagonist
potency in vitro on human PMNs for compounds **15** and **17** with
those for compound **7**. Both **15** and **17** are potent inhibitors
25   at nM concentrations of the action of C5a and the binding
of ^{125}I-C5a to its receptor (e.g. 4, K_b = 1.4nM). Their
cyclic nature and the acetylation at the N-terminal
phenylalanine both protect against the proteolytic
degradation typically encountered by peptides, making such
30   cyclic compounds more suitable than acyclic peptides as
drug candidates. The results are shown in Table 5.

Table 5

Receptor Binding and Antagonist Activity of Cyclic Molecules
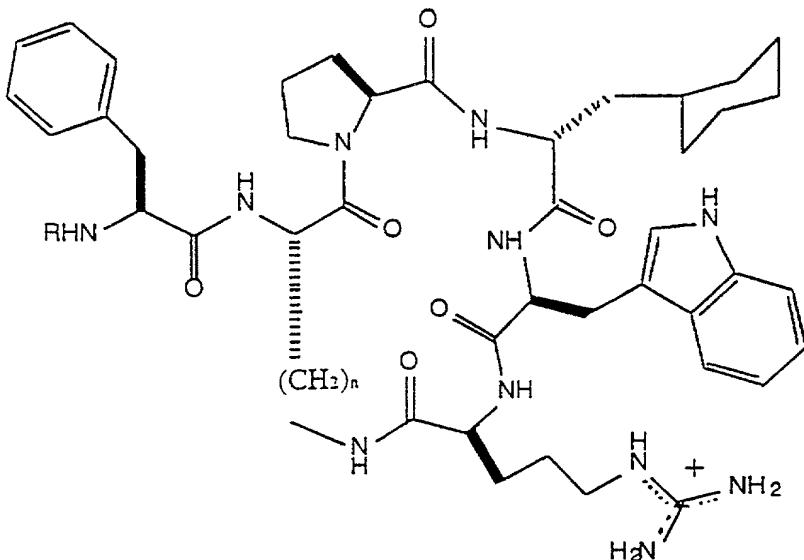
Compound	n	R	Isomer	Receptor Affinity μM	Agonist Activity
13	1	H	S-	9	No
14			R-	34	No
15	2	H	S-	0.3	No
16			R-	3.7	No
17	3	Ac	S-	0.3	No
11		Ac	R-	38	No
18	4	Ac	S-	3.2	No
12		Ac	R-	51	No

Refers to stereochemistry of Arg side chain.

- **40** -

Example 4   Cyclic Antagonists of C5a

    Some examples of these cyclic antagonists and
their apparent receptor-binding affinities and antagonist
potencies are given in Tables 4, 5 and 6 as well as in

5   Figures 5 and 6. In the tables the single letter code for
amino acids is used.

Table 6

Effect of Cyclisation on Antagonist Binding Affinity and Antagonist Potency

	PEPTIDE	pD2 ± SE[a]	IC50 (µM)[a]	(n)	pD2 ± SE[b]	IC50 (µM)[b]	(n)
11	AcF-[KPdChawR]	5.49 ± 0.22	3.2	4	7.07 ± 0.29	0.09	5
18	AcF-[OPdChawR]	6.44 ± 0.14*	0.4	9	7.30 ± 0.09	0.05	9
19	[FWPdChawR]	4.37 ± 0.36*	43	3	nd		
20	AcF-[KMdChawR]	4.81 ± 0.06	15	2	nd		
21	AcF-[KKdChawR]	3.94 ± 0.4	116	3	4.88	13	1

Effect of length of linker in cycle on antagonist binding affinity and antagonist potency

	PEPTIDE	pD2 ± SE[a]	IC50 (µM)[a]	(n)	pD2 ± SE[b]	IC50 (µM)[b]	(n)
22	AcF-[XPdChawR]	5.02 ± 0.07	9.5	3	4.71 ± 0.23	20	3
23	AcF-[X^{2}PdChawR]	4.77 ± 0.14*	17	3	6.09 ± 0.08*	0.8	4
11	AcF-[OPdChawR]	4.60±0.06*	16	4	6.42 ± 0.10	0.4	4
24	AcKF-[OPdChawR]	4.96 ± 0.03	11	3	6.73	0.2	1

Table 6 (cont.)

	PEPTIDE	pD2 ± Se[a]	IC50 (µM)[a]	(n)	pD2 ± SE[b]	IC50 (µM)[b]	(n)
14	F-[XPdChaWR]	4.39 ± 0.10*	41	3	nd		
16	F-[X²PdChaWR]	5.42 ± 0.05	3.8	3	6.70 ± 0.04	0.4	3
25	F-[OPdChaWR]	5.51 ± 0.07	3.1	3	5.79 ± 0.34*	1.6	3
26	F-[KPdChaWR]	5.09 ± 0.08	8.1	3	5.55 ± 0.57*	2.8	3
	Effect of L-Arg on antagonist binding affinity and antagonist potency						
17	AcF-[OPdChaWR]	6.57 ± 0.05*	0.3	3	7.91 ± 0.17*	0.01	3
13	F-[XPdChaWR]	4.98 ± 0.05	10	3	5.63 ± 0.13*	2.4	3
15	F-[X²PdChaWR]	6.50 ± 0.04*	0.3	5	7.36 ± 0.13	0.04	3
27	F-[OPdChaWR]	7.21 ± 0.01*	0.06	3	7.41 ± 0.14	0.04	3
28	F-[KPdChaWR]	6.50 ± 0.12*	0.3	4	6.69 ± 0.04	0.2	3

a pD_2/IC_{50}; concentration of peptide resulting in 50%
     inhibition in the binding of $[^{125}I]C5a$ to intact PMNs.
     The IC50 is the antilog of the mean pD2 value

b pD_2/IC_{50}; concentration of peptide resulting in 50%
5   inhibition in the ability of C5a (100 nM)to cause the
     release of MPO from PMNs

X = $(CH_2)-NH_2$

X^2 = $(CH_2)_2-NH_2$

pD2 values are expressed as mean ± SE

10   n represents the number of experiments performed

*   Significant change in affinity/potency compared to
     NMeFKPdChaWR ($p<0.05$)

indicates isomer number

15   These results demonstrate:

          (1)   that the cyclic molecules have higher
     apparent receptor affinity and may be more potent
     antagonists than acyclic (linear) peptides,

          (2)   that one of the two possible cyclic
20   diastereomers is consistently favoured for binding to the
     C5a receptor, and it is surprisingly the opposite
     stereochemistry (L-arginine) to that favoured in the linear
     compounds (D-arginine)

          (3)   that the cycles have an optimum ring size
25   for receptor-binding,

          (4)   that there is a pseudo-linear relationship
     between log (antagonist potency) and log (receptor
     affinity).

          Tables 5 and 6 list the C5a receptor affinities
30   of some examples of cyclic antagonists of C5a, and their
     ability to bind to, and inhibit, binding of C5a to human
     PMNs is illustrated in Figure 6. Surprisingly these data
     show that the L-arginine is preferred over the D-arginine,
     in contrast to the linear compound **7** in which the D-
35   arginine confers higher affinity for the receptor than does
     L-arginine. The data also show that the size of the
     macrocycle is optimal when n = 2 or 3, the smaller cycle

- 44 -

where n = 1 and the larger cycle when n = 4 being clearly
less active. This requirement for a tightly constrained
cycle is probably due to the need to correctly position the
attached side chain residues of, for example, Trp, dCha,
5   Arg and Phe for interaction with the receptor.

Example 5   Computer Modelling of Antagonist Structures
        Figure 7 compares the computer-modelled structure
of the cyclic antagonist **12** with the NMR solution structure
10   for the acyclic antagonist **7**. These backbone structures
are strikingly similar, and strongly suggest that the
receptor-binding conformations of these molecules involve
the same turn structure. Compound **12**, a more potent
antagonist than **11**, also has a shorter linker, which
15   tightens the turn and slightly alters the conformational
space accessible to the key side chains of Phe, dCha, Trp
and Arg. The conformational limitations placed on the
hexapeptide derivative **12** by the cycle are responsible for
a $\geq 10^4$ increase in receptor-binding affinity over the
20   conformationally flexible decapeptide C-terminus of C5a (**1**,
Table 2).
        There is a correlation between binding affinities
and antagonist potency for the site 2 antagonists
(compounds **7-12**, Table 2). It thus appears that antagonist
25   potency is dependent upon changes that occur at site 2
alone. Without wishing to be bound by any proposed
mechanism, we believe that this may be because the
mechanism of antagonism is related to conformational change
to a turn conformation induced by **7** at site 2 of the
30   receptor.

Example 6 Characterisation of C5aRs on Different Cells
        Currently there is no information about different
types of C5aRs. We have previously shown marked
35   differences in the responsiveness of different cells
containing functional C5aRs to agonists (Sanderson *et al*,
1994, 1995; Finch *et al*, 1997) and we can now provide more

- 45 -

information by examining potency and efficacy of selective
agonists and antagonists relative to human recombinant C5a.
For agonists, the tissue or cell selectivity may reveal
functionally different receptors. Binding assays using
5   human PMNs, U937 cells, or circulating monocytes are used
to determine affinities for C5aRs. Selectivity for
different C5aRs is ascertained by differential antagonism.
This combined approach allows pharmacological
characterisation of new agonists or antagonists, and may
10   lead to a potential functional classification of C5aRson
different cells.

Example 7(a)   Neutropenia and C5a Antagonism _In Vivo_
              Compounds were evaluated in an acute model of
15   C5a-induced neutropenia. Transient neutropenia maximises 5
min after i.v. C5a and is profound, with >90% of
circulating neutrophils disappearing from circulation at
effective doses of C5a, as shown in Figure 8. The
neutropenia is due to transient adherence of circulating
20   neutrophils to the vascular endothelium.   Preliminary
data show that neutropenia caused by i.v. C5a is blocked
by a C5a antagonist. For example, F-[OPdChaWR], (1 mg/kg),
given prior to 2µg C5a i.v., inhibits C5a-induced
neutropenia _in vivo_ (Figure 8).
25

Example 7(b)   Inhibition of Lipopolysaccharide-induced
                Effects by C5a Antagonists
              LPS causes rapid neutropenia in rats. If this
effect of LPS is blocked by C5a antagonists, then C5a may
30   be of major importance in the acute effects of LPS, and the
results shown in Figure 9 were in agreement with this
hypothesis. C5a antagonists were injected (bolus i.v.) 10
min prior to challenge with LPS. Rats were anaesthetised,
and blood samples (0.3ml) were taken for measurements of
35   PMNs. PMNs are isolated and quantified. Preliminary results
show that F-[OPdChaWR], (1 mg/kg), given prior to i.v.LPS,
inhibits neutropenia..

- 46 -

The results also indicate that the C5a antagonist inhibits the increase in haematocrit caused by LPS, showing that vascular leakage of serum caused by LPS is also inhibited.

5   These results demonstrate that C5a receptor antagonists, such as those described in this invention, may have therapeutic utility in septicaemic individuals. The ability to inhibit the adherence of PMNs to vascular endothelium, and to inhibit the vascular leakage to LPS as

10   shown by the reduction of haematocrit values, indicates powerful anti-inflammatory effects of these compounds against proinflammatory stimuli activating the complement system, such as endotoxin or LPS.

15   Example 8   _In Vivo Activity of Cyclic C5a Antagonists_
           Preliminary experiments in rats have revealed that the cyclic antagonists summarized in Table 5 are active at less than 20 mg/kg as anti-inflammatory agents in suppressing the onset of either carrageenan-induced paw

20   oedema or adjuvant-induced polyarthritis. The maximally effective dosages for even moderately-effective antagonists are 10 mg/kg or less, given i.p. or p.o. Many anti-inflammatory drugs currently used in humans were initially evaluated in such assays, and also showed activity in these

25   rat models of inflammation. These preliminary indications of efficacy _in vivo_ indicate that C5a antagonists have therapeutic potential in human inflammatory conditions.
           Using the rat carageenan paw oedema assay, we found that a compound, AcF-[O-P-dCha-W-r], which is

30   100 times less active than **17** _in vitro_ as a C5a antagonist in PMNs, has some _in vivo_ activity in rats given 1 mg/kg of the compound I.P, 30 min prior to the carageenan injection. Paw swelling was measured for up to 4.5 hr. The results, shown in Figure 10, suggest that even this weak C5a

35   antagonist significantly inhibits development of the oedema after 180 and 270 min. This anti-inflammatory activity suggests that C5a receptor antagonists, such as those

- 47 -

described in this invention, may have therapeutic activity
in diseases involving vascular leakage following
inflammatory stimuli.

5   In recent years there have been many attempts to
mimic β- and γ- turn peptides that represent bioactive
protein surfaces, resulting in notable mimetics for RGD
(arginine-glycine-aspartate) peptides, somatostatin and
opioid peptides, to name a few derived through structure-
activity relationships (see for example Marraud and Aubry,
10   1996; Fairlie et al, 1995). Most of these examples
preserve a turn structure through cyclisation of the
peptide.   On the other hand, there are comparatively few
short acyclic peptides that have been found to have
substantial turn structure in solution (Dyson et al, 1988;
15   Rizo and Gierasch, 1992; Pràcheur et al, 1994).   It is
usually argued that short acyclic peptides adopt a myriad
of solution structures that may include small populations
of turn structures that are responsible for bioactivity.

          This invention describes a series of
20   conformationally-constrained turn-containing molecules that
are preorganized for binding to the same G protein-coupled
receptor(s) of human cells that are targeted by human C5a.
The invention is applicable to other G protein-coupled
receptors.

25   The principal feature of the compounds of the
invention is the preorganized arrangement, which brings at
least three hydrophobic groups and a charged group into
neighbouring space, creating a hydrophobic surface 'patch'.
These results enable the design and development of even
30   more potent conformationally-constrained, small molecule
antagonists of C5a.

          In the light of the aforementioned prior art, it
was surprising to find that a C-terminal carboxylate was
not necessary in our compounds in order to obtain good
35   receptor-binding or antagonist activity. The cyclic
antagonists have an amide bond at the 'C-terminal' arginine
position. The replacement of the carboxylate in 7 with a

covalent amide bond effectively stabilises the required
turn conformation.

5   Cyclic and non-peptidic antagonists have several
important advantages over peptides as drugs. The cycles
described in this invention are stable to proteolytic
degradation for at least several hours at 37°C in human
blood or plasma, or in human or rat gastric juices or in
the presence of digestive enzymes such as pepsin, trypsin
10   and chymotrypsin. In contrast, short peptides composed of
L-amino acids are rapidly degraded to their component amino
acids within a few minutes under these conditions. A
second advantage lies in the constrained single
conformations adopted by the cyclic and non-peptidic
15   molecules, whereas acyclic or linear peptides are flexible
enough to adopt several structures in solution other than
the required receptor-binding structure. Thirdly, cyclic
and non-peptidic compounds such as those described in this
invention are usually more lipid-soluble and more
20   pharmacologically bioavailable as drugs than peptides,
which can rarely be administered orally. Fourthly, the
plasma half-lives of cyclic and non-peptidic molecules are
usually longer than those of peptides.

25   It will be apparent to the person skilled in the
art that while the invention has been described in some
detail for the purposes of clarity and understanding,
various modifications and alterations to the embodiments
and methods described herein may be made without departing
30   from the scope of the inventive concept disclosed in this
specification.

References cited herein are listed on the
following pages, and are incorporated herein by this
35   reference.

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<u>CLAIMS</u>:

1.   A compound which is an antagonist of a G protein-coupled receptor, which has no agonist activity, and which has a cyclic on constrained acyclic structure adapted to

5   provide a framework of approximate dimensions as set out in Structure I:

Structure I
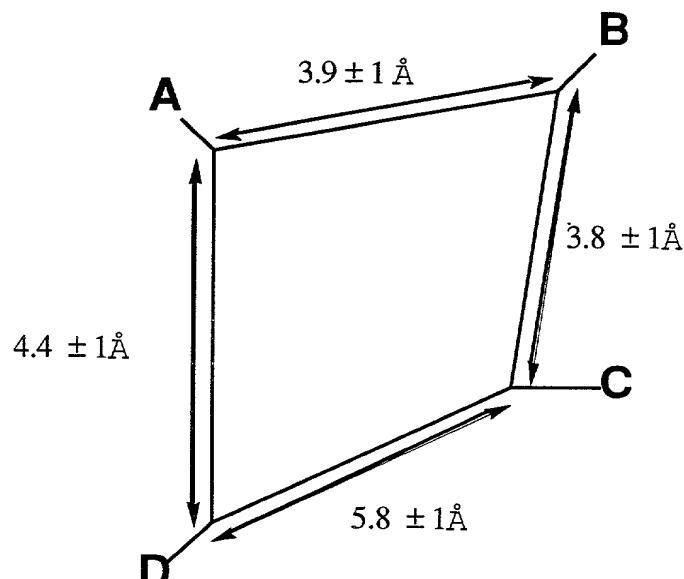
10

where the numerals refer to distances between C_α carbons of amino acids or their analogues or derivatives, and A, B, C and D are not necessarily on adjacent amino

15   acids, or analogues or derivatives thereof; and

where the critical amino acid side chains are designated by A, B, C and D, where

A is any common or uncommon, basic, charged amino acid side chain which serves to position a positively

20   charged group in this position;

B is any common or uncommon, aromatic amino acid side chain which serves to position an aromatic side-chain in this position;

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AMENDED SHEET (Article 34) (IPEA/AU)

- 55 -

C is any common or uncommon, hydrophobic amino acid side chain which serves to position any alkyl, aromatic or other group in this position;

D is any common or uncommon, aromatic amino acid which serves to position an aromatic side-chain in this position, and has the structure:
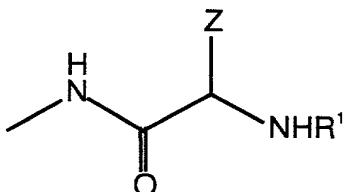

where   Z is indole, indole methyl, benzyl, benzene, naphthyl, naphthyl methyl, or a derivative thereof; and

R^1 is H or an alkyl, aromatic, acyl or aromatic-acyl group.

2.   An antagonist according to Claim 1, in which the G protein-coupled receptor is the C5a receptor.

3.   An antagonist according to Claim 1 or Claim 2, in which

A is one of the following side-chains
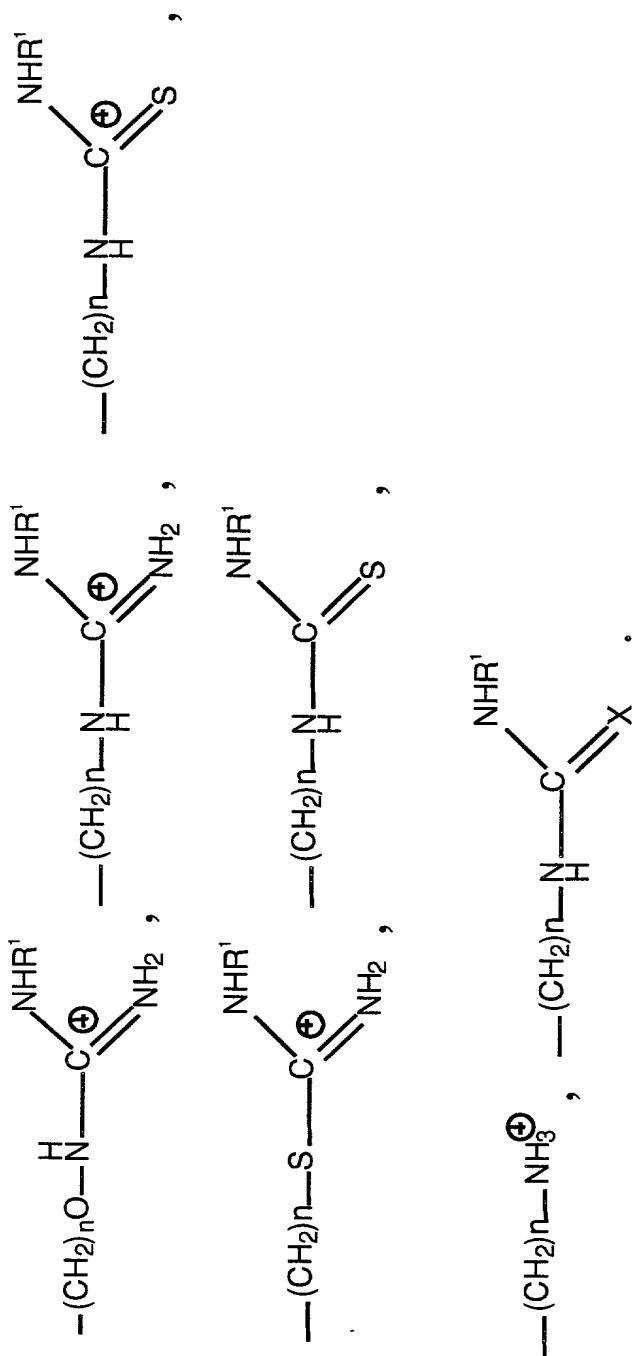
or another mimetic of an arginine side chain;
where

X is NCN, NNO$_2$, CHNO$_2$ or NSO$_2$NH$_2$;

n is an integer from 1 to 4, and

5   R^1 is H or an alkyl, aryl, CN, NH$_2$, OH, -CO-CH$_2$CH$_3$, -CO-CH$_3$, -CO-CH$_2$CH$_2$CH$_3$, -CO-CH$_2$Ph, or -CO-Ph;

B is an indole, indole methyl, benzyl, phenyl, naphthyl, naphthyl methyl, cinnamyl group, or any other derivative of

10   the aromatic group; and

C is D- or L-cyclohexylalanine (Cha), leucine, valine, isoleucine, phenylalanine, tryptophan or methionine.

4.   An antagonist according to Claim 3, in which

15   R^1 is methyl, ethyl, propyl, or butyl.

5.   An antagonist according to any one of Claims 1 to 4, which is a constrained acyclic compound, and comprises a type II β-turn.

6.   An antagonist according to Claim 5, in which the

20   type II β-turn comprises a γ-turn within the type II β-turn.

7.   An antagonist according to any one of claims 1 to 4, which is a cyclic peptide or peptide derivative.

8.   An antagonist according to any one of Claims 1 to

25   4, of formula

Ac-phe-[lys-pro-(dCha)-trp-arg] or

Ac-phe-[orn-pro-(dCha)-trp-arg]

9.   An antagonist according to any one of Claims 1 to 7 in which A is L-arginine.

30   10.   An antagonist according to Claim 1, which has antagonist activity against C5aR, has no agonist activity against C5a, and has the general formula:

Structure II
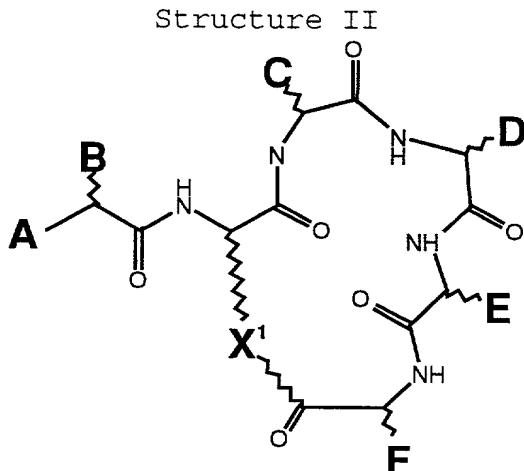
where   A is H, alkyl, aryl, NH$_2$, NHalkyl, N(alkyl)$_2$,
5   NHaryl or NHacyl;

B is an alkyl, aryl, phenyl, benzyl, naphthyl or
indole group, or the side chain of a D- or L-amino acid
selected from the group consisting of phenylalanine,
homophenylalanine, tryptophan, homotryptophan, tyrosine,
10   and homotyrosine;

C is the side chain of a D-, L- or homo-amino
acid selected from the group consisting of proline,
alanine, leucine, valine, isoleucine, arginine, histidine,
aspartate, glutamate, glutamine, asparagine, lysine,
15   tyrosine, phenylalanine, cyclohexylalanine, norleucine,
tryptophan, cysteine and methionine;

D is the side chain of a D- or L-amino acid
selected from the group consisting of cyclohexylalanine,
homocyclohexylalanine, leucine, norleucine, homoleucine,
20   homonorleucine and tryptophan;

E is the side chain of a D- or L-amino acid
selected from the group consisting of tryptophan and
homotryptophan;

F is the side chain of a D- or L-amino acid
25   selected from the group consisting of arginine,
homoarginine, lysine and homolysine; and

X^1 is $-(CH_2)_nNH-$ or $(CH_2)_n-S-,-(CH_2)_2O-,$ $-(CH_2)_3O-,$ $-(CH_2)_3-,$ $-(CH_2)_4-,$ or $-CH_2COCHRNH-,$ where R is the side chain of any common or uncommon amino acid, and

where n is an integer of from 1 to 4,

5   11.   An antagonist according to Claim 10, in which F is a L-amino acid.

12.   An antagonist according to Claim 11, in which F is L-arginine.

13. An antagonist according to any one of Claims 10 to 12,

10   selected from the group consisting of compounds **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **26**, **27** and **28**.

14.   An antagonist according to any one of Claims 3 and 10 to 13, in which n is 2 or 3.

15   15.   A compound which is an agonist of a G protein-coupled receptor, and which has structure III
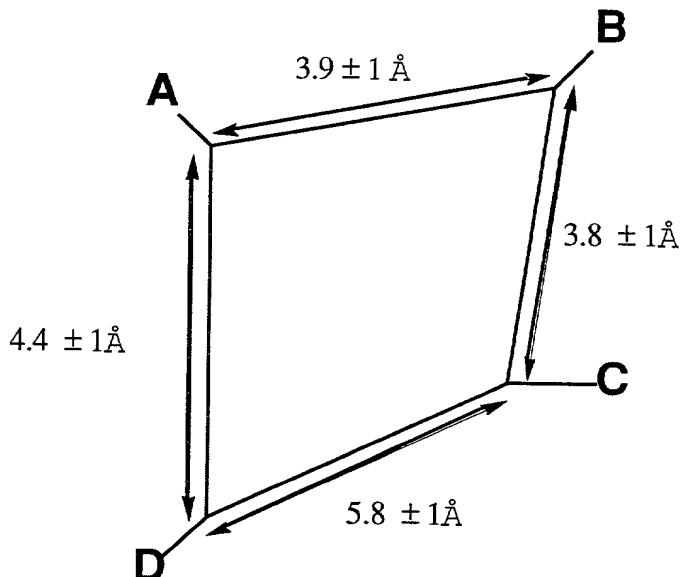
Structure III

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where the numerals refer to distances between C_α carbons of amino acids or their analogues or derivatives,

**AMENDED SHEET (Article 34) (IPEA/AU)**

and A, B, C and D are not necessarily on adjacent amino acids, or analogues or derivatives thereof; and

where B is a non-aromatic amino acid, and

5   A is any common or uncommon, basic, charged amino acid side chain which serves to position a positively charged group in this position;

C is any common or uncommon, hydrophobic amino acid side chain which serves to position any alkyl, aromatic or other group in this position; and

10   D is any common or uncommon, aromatic amino acid which serve to position an aromatic side-chain in this position, and has the structure:
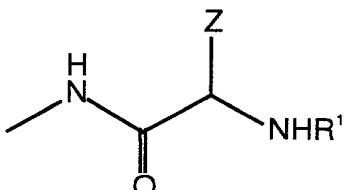
15

where   Z is indole, indole methyl, benzyl, benzene, naphthyl, naphthyl methyl, or a derivative thereof; and

20   R is H or an alkyl, aromatic, acyl or aromatic-acyl group.

16.   A compound according to Claim 15, where B is the D- or L-form of alanine, leucine, valine, norleucine, glutamic acid, aspartic acid, methionine, cysteine,

25 isoleucine, serine or threonine.

17.   A compound according to Claim 15 or Claim 16, in which the compound is of structure IV,

Structure IV
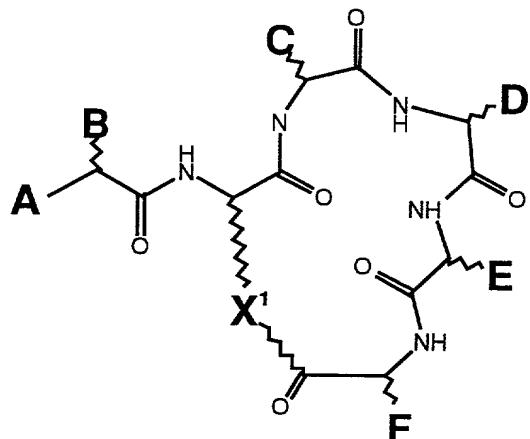
where E is any amino acid other than tryptophan and homotryptophan, and

F is the side chain of a D- or L-amino acid selected from the group consisting of arginine, homoarginine, lysine and homolysine.

18.   A compound according to any one of Claims 15 to 17, wherein the compound is an agonist of C5a.

19.   A compound according to Claim 10, of structure
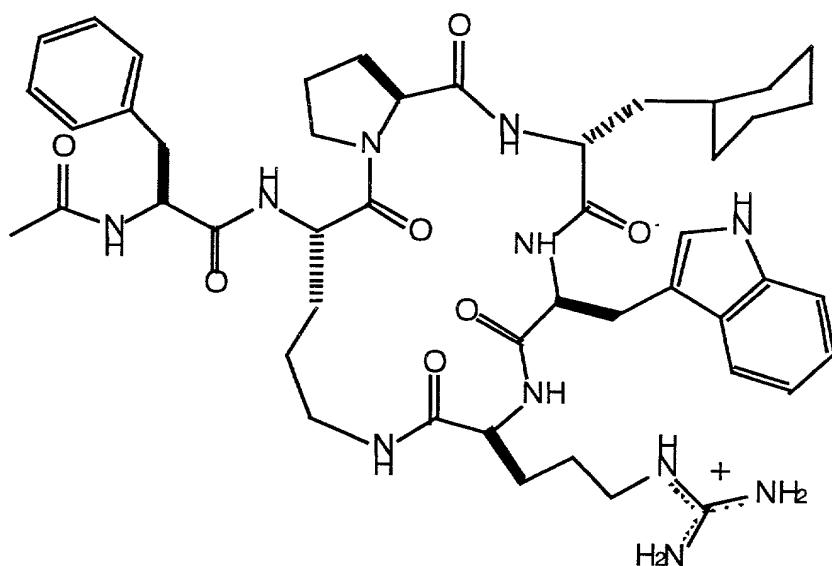
20.   A composition comprising a compound according to any one of Claims 1 to 19, together with a pharmaceutically-acceptable carrier or excipient.

21.   A method of treatment of a pathological condition
5   mediated by a G protein-coupled receptor, comprising the step of administering an effective amount of a compound according to any one of Claims 1 to 19, to a mammal in need of such treatment.

22.   A method according to Claim 21, wherein the
10   condition mediated by a G protein-coupled receptor involves overexpression or underregulation of C5a.

23.   A method according to Claim 21, wherein the condition is selected from the group consisting of rheumatoid arthritis, adult respiratory distress syndrome
15   (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, multiple sclerosis, lung injury and extracorporeal post-dialysis syndrome.

20   24.   Use of a compound according to any one of Claims 1 to 19 in treatment of a pathological condition mediated by a G protein-coupled receptor.

25.   Use according to Claim 24, in which the condition is mediated by C5a.

25   26.   Use according to Claim 25, in which the condition mediated by G protein-coupled receptors involves overexpression or underregulation of C5a.

27.   Use according to any one of Claims 24 to 26, in which the condition is selected from the group consisting
30   of rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, multiple sclerosis, lung injury and
35   extracorporeal post-dialysis syndrome.

28.   Use of a compound according to any one of Claims 1 to 19 in the manufacture of a medicament for the

treatment of a condition mediated by a G protein-coupled receptor.

29.   Use according to Claim 28, in which the condition is mediated by C5a.

5   30.   Use according to Claim 29, in which the condition mediated by G protein-coupled receptors involves overexpression or underregulation of C5a.

31.   A compound according to Claim 1, substantially as hereinbefore described with reference to the examples and drawings.

10   32.   A method according to Claim 21, substantially as hereinbefore defined with reference to the examples and drawings.
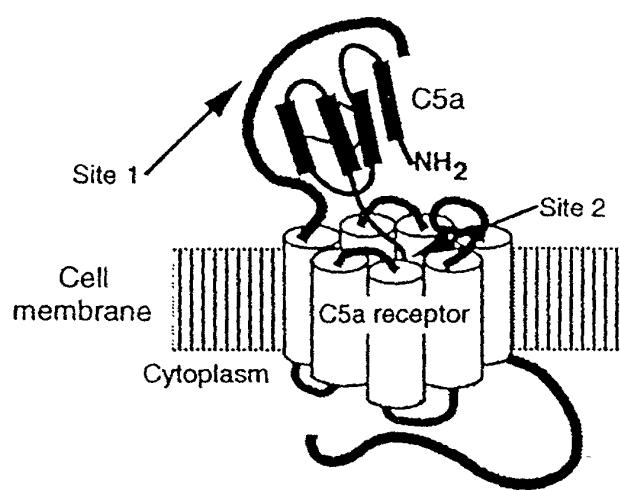
Fig. 1
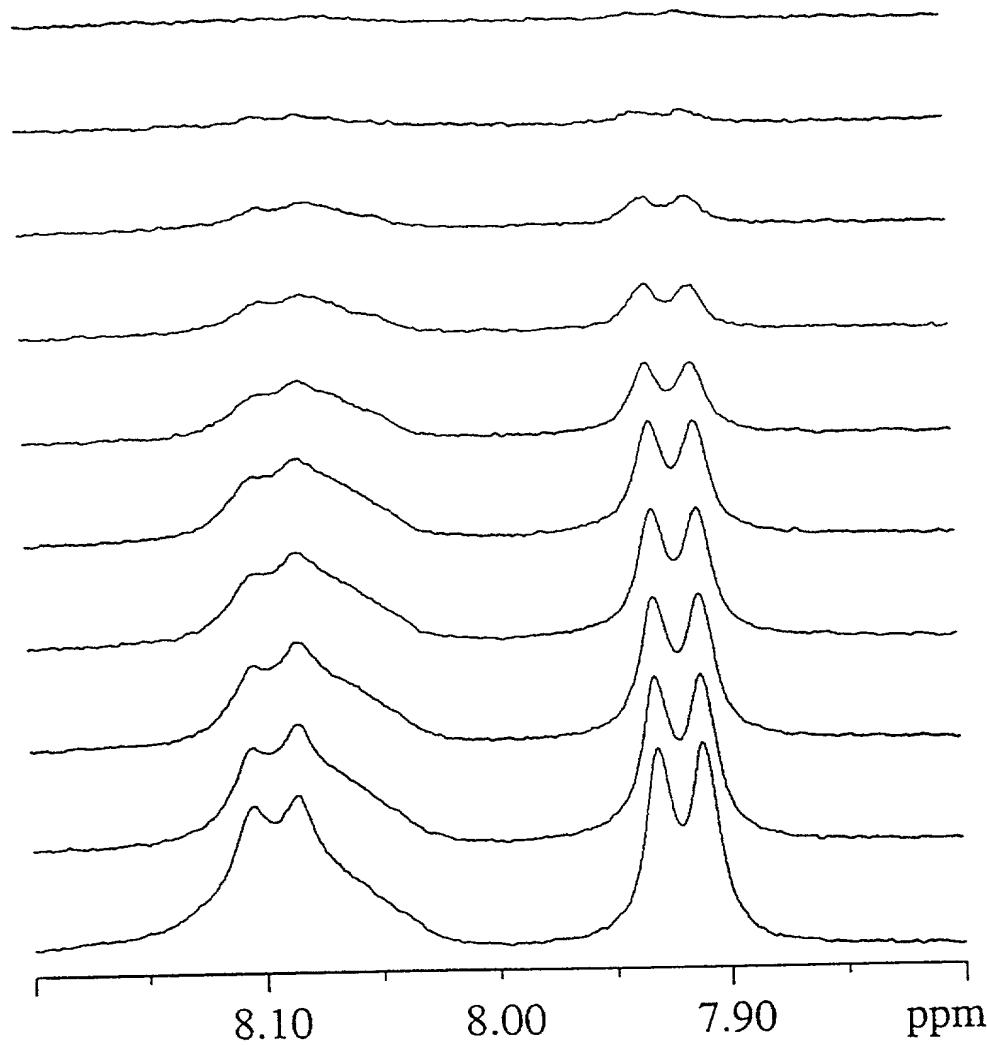
Fig. 2
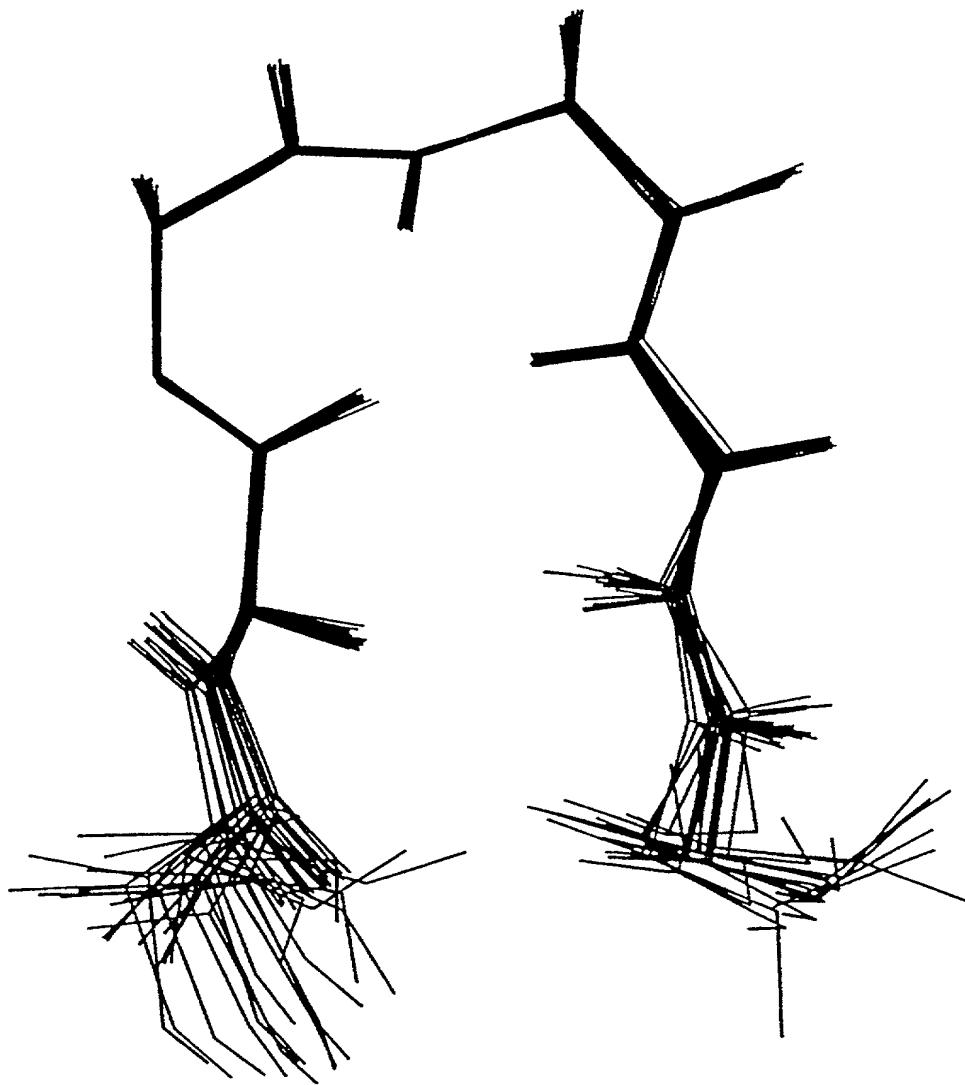
Fig. 3

Fig. 4

Fig. 5a

Fig. 5 b

Fig. 5c

Fig. 6

dCha

Pro

Lys/Orn

dArg

Fig. 7

Fig. 8

A

B
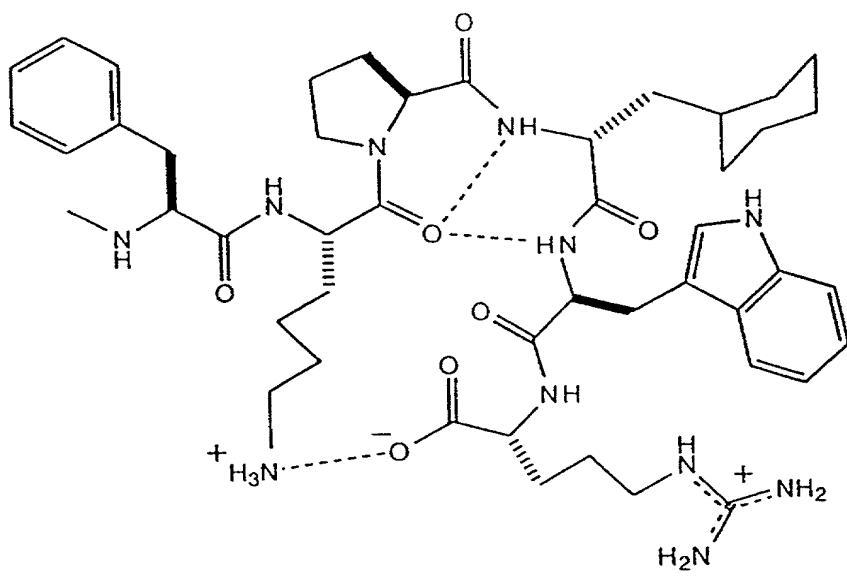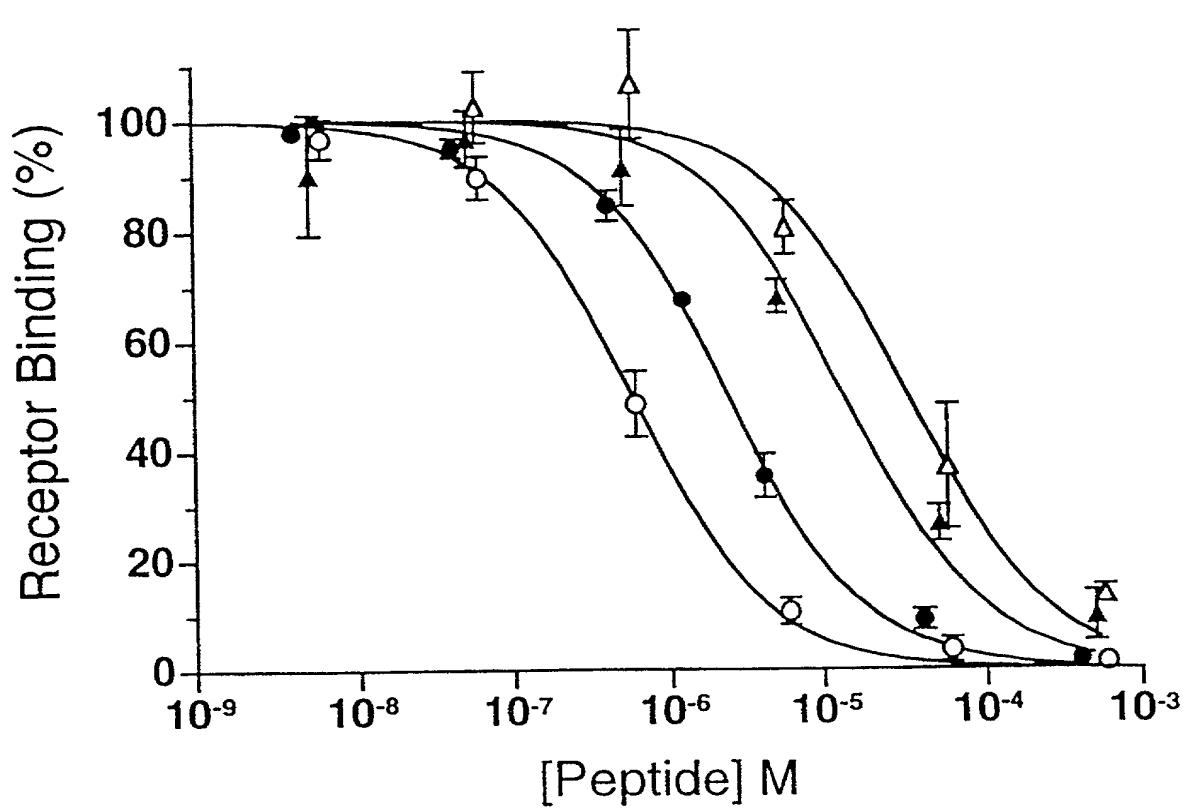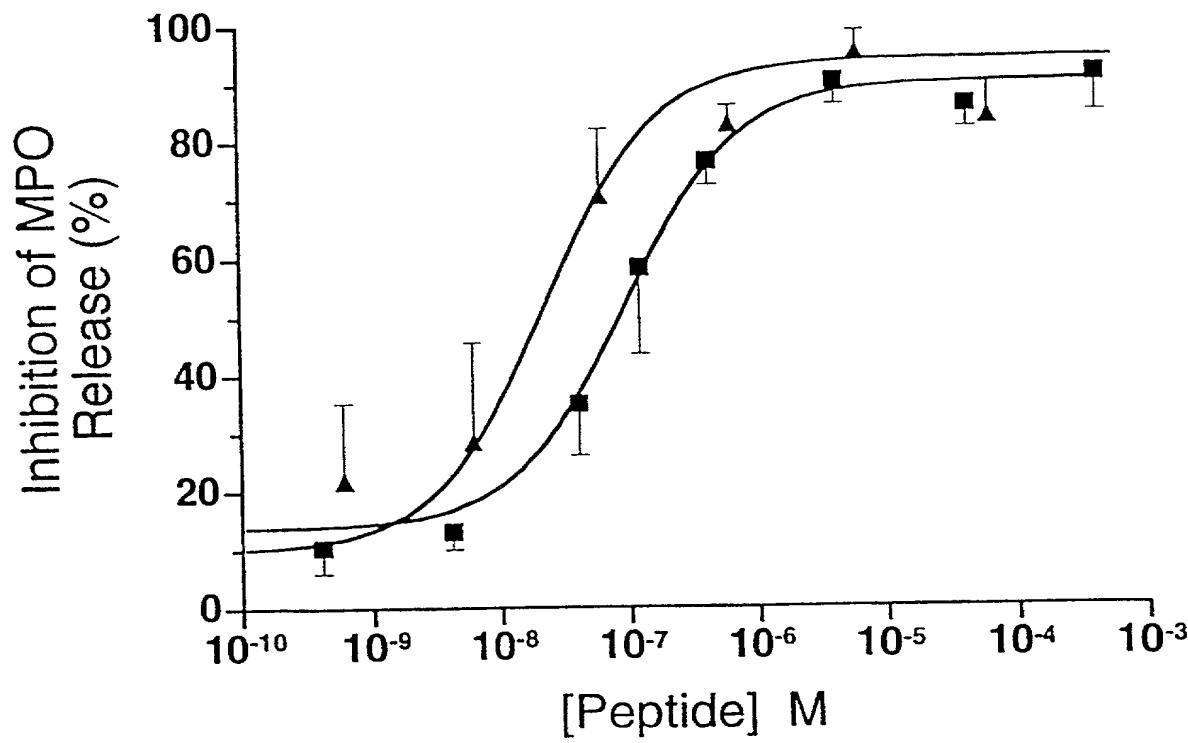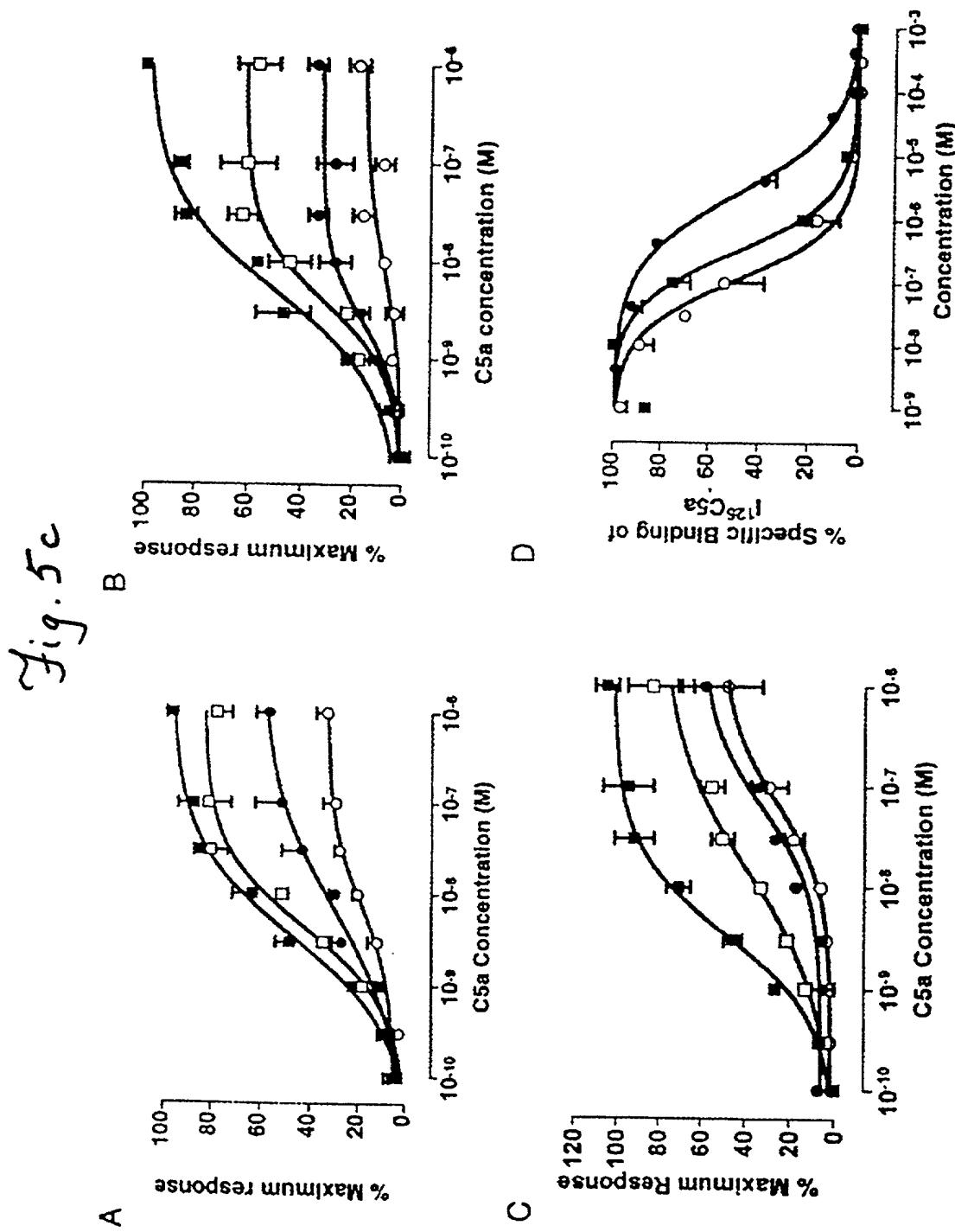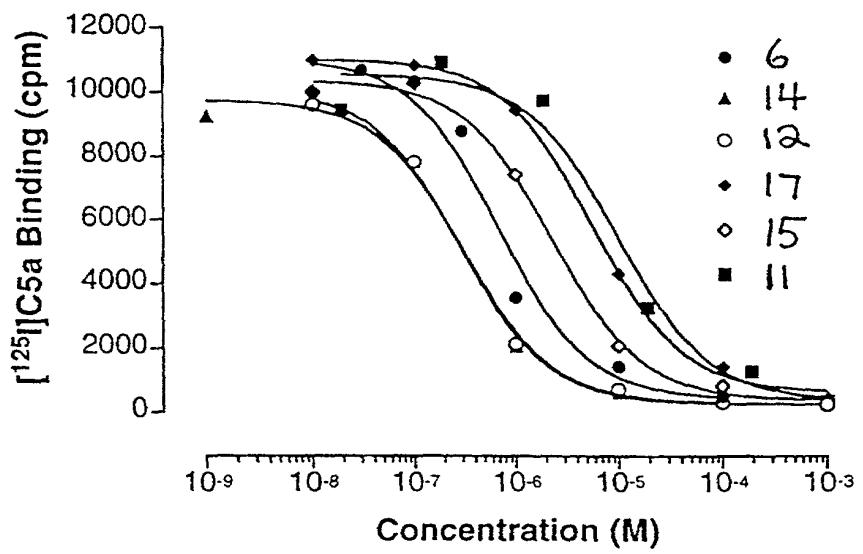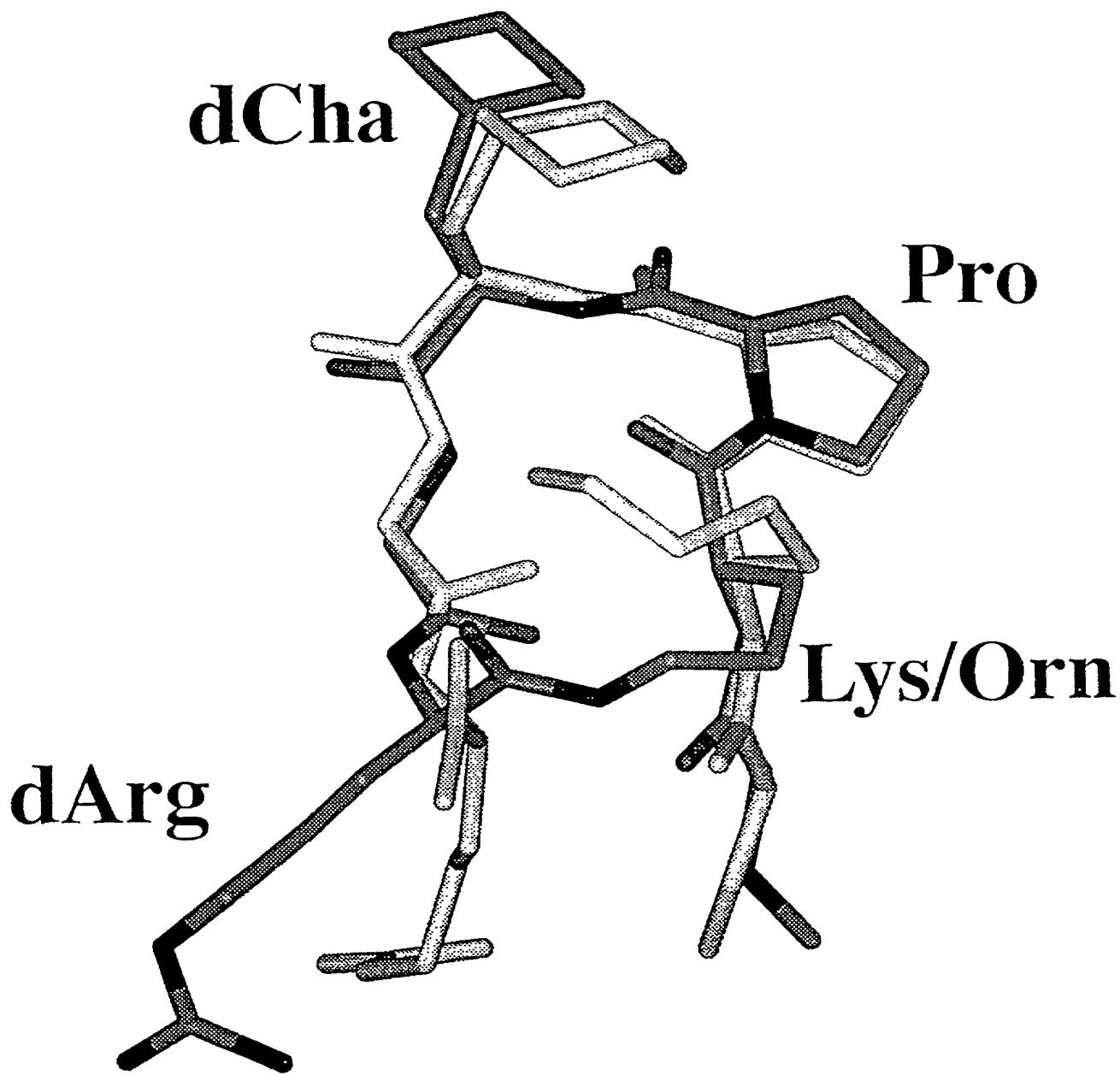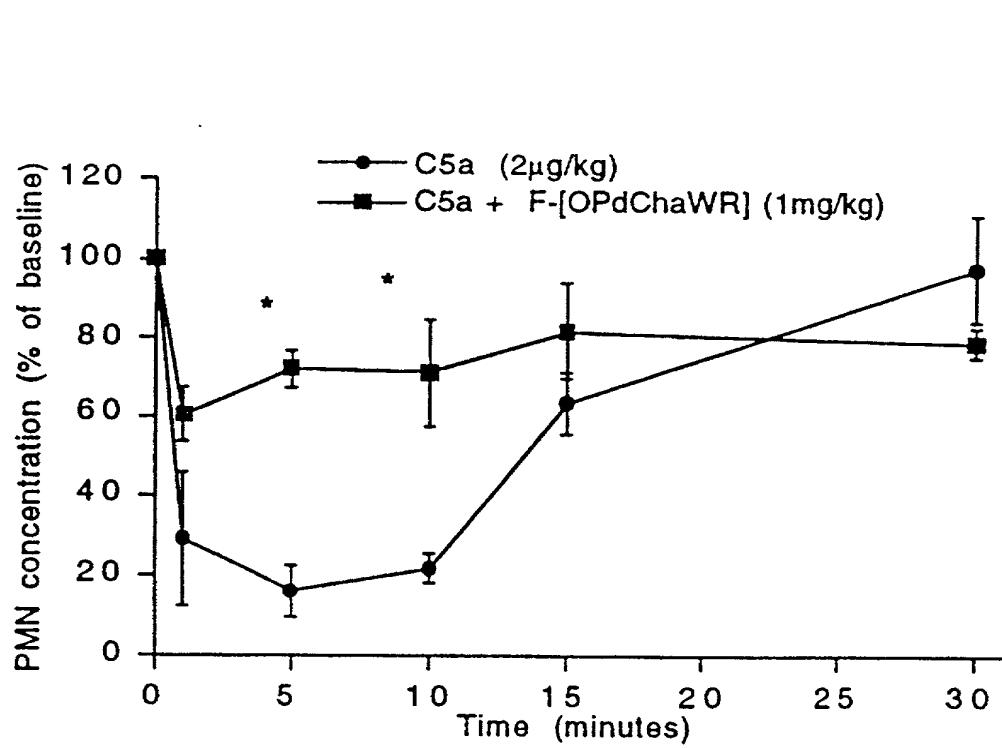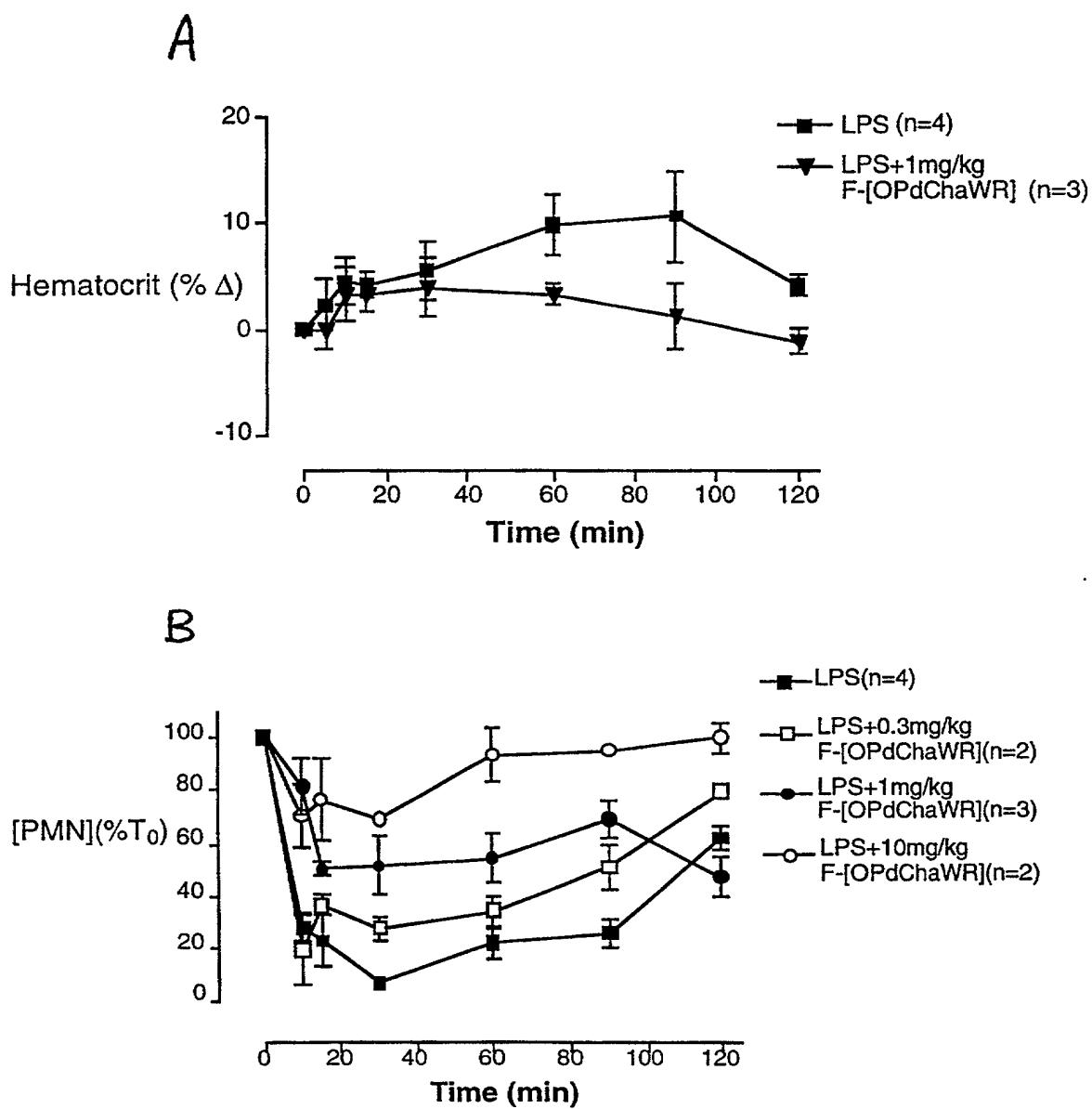
Fig. 9
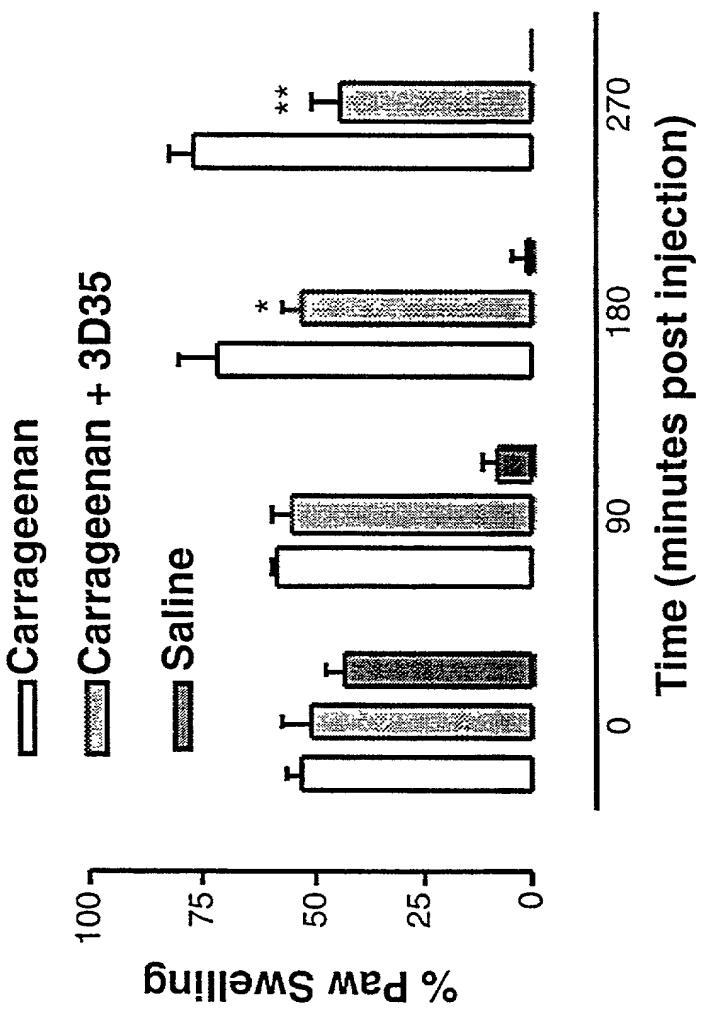
Fig. 10

# Declaration, Power Of Attorney and Petition

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

CYCLIC AGONISTS AND ANTAGONISTS OF C5a RECEPTORS AND G PROTEIN-COUPLED RECEPTORS

the specification of which

    ☐ is attached hereto.

    ☒ was filed on ___December 23, 1999___ as

    Application Serial No. ___09/446,109___

    and amended on _____ .

    ☒ was filed as PCT international application

    Number _PCT/AU98/00490_

    on ___25 June 1998___ ,

    and was amended under PCT Article 19

    on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed	
PO 7550	AUSTRALIA	25 June 1997	☒ Yes	☐ No
_____	_____	_____	☐ Yes	☐ No
_____	_____	_____	☐ Yes	☐ No
_____	_____	_____	☐ Yes	☐ No

₀ We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)
PCT/AU98/00490	25 June 1998	

And we (I) hereby appoint: Norman F. Oblon, Reg. No. 24,618; Marvin J. Spivak, Reg. No. 24,913; C. Irvin McClelland, Reg. No. 21,124; Gregory J. Maier, Reg. No. 25,599; Arthur I. Neustadt, Reg. No. 24,854; Richard D. Kelly, Reg. No. 27,757; James D. Hamilton, Reg. No. 28,421; Eckhard H. Kuesters, Reg. No. 28,870; Robert T. Pous, Reg. No. 29,099; Charles L. Gholz, Reg. No. 26,395; Vincent J. Sunderdick, Reg. No. 29,004; William E. Beaumont, Reg. No. 30,996; Robert F. Gnuse, Reg. No. 27,295; Jean-Paul Lavalleye, Reg. No. 31,451; Stephen G. Baxter, Reg. No. 32,884; Robert W. Hahl, Reg. No. 33,893; Richard L. Treanor, Reg. No. 36,379; Steven P. Weihrouch, Reg. No. 32,829; John T. Goolkasian, Reg. No. 26,142; Richard L. Chinn, Reg. No. 34,305; Steven E. Lipman, Reg. No. 30,011; Carl E. Schlier, Reg. No. 34,426; James J. Kulbaski, Reg. No. 34,648; Richard A. Neifeld, Reg. No. 35,299; J. Derek Mason, Reg. No. 35,270; Surinder Sachar, Reg. No. 34,423; Christina M. Gadiano, Reg. No. 37,628; Jeffrey B. McIntyre, Reg. No. 36,867; Paul E. Rauch, Reg. No. 38,591; William T. Enos, Reg. No. 33,128; and Michael E. McCabe, Jr., Reg. No. 37,182; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C., whose Post Office Address is: Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

David FAIRLIE

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Signature of Inventor

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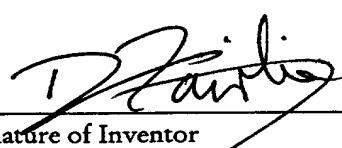
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Signature of Inventor
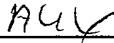
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Signature of Inventor
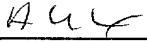
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NAME OF FIFTH JOINT INVENTOR

Signature of Inventor
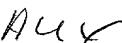
Date

Residence:

Citizen of:

Post Office Address: